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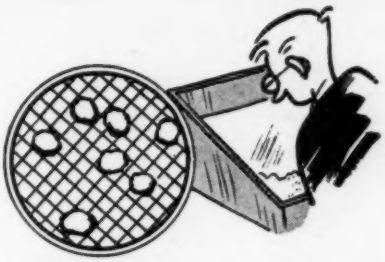

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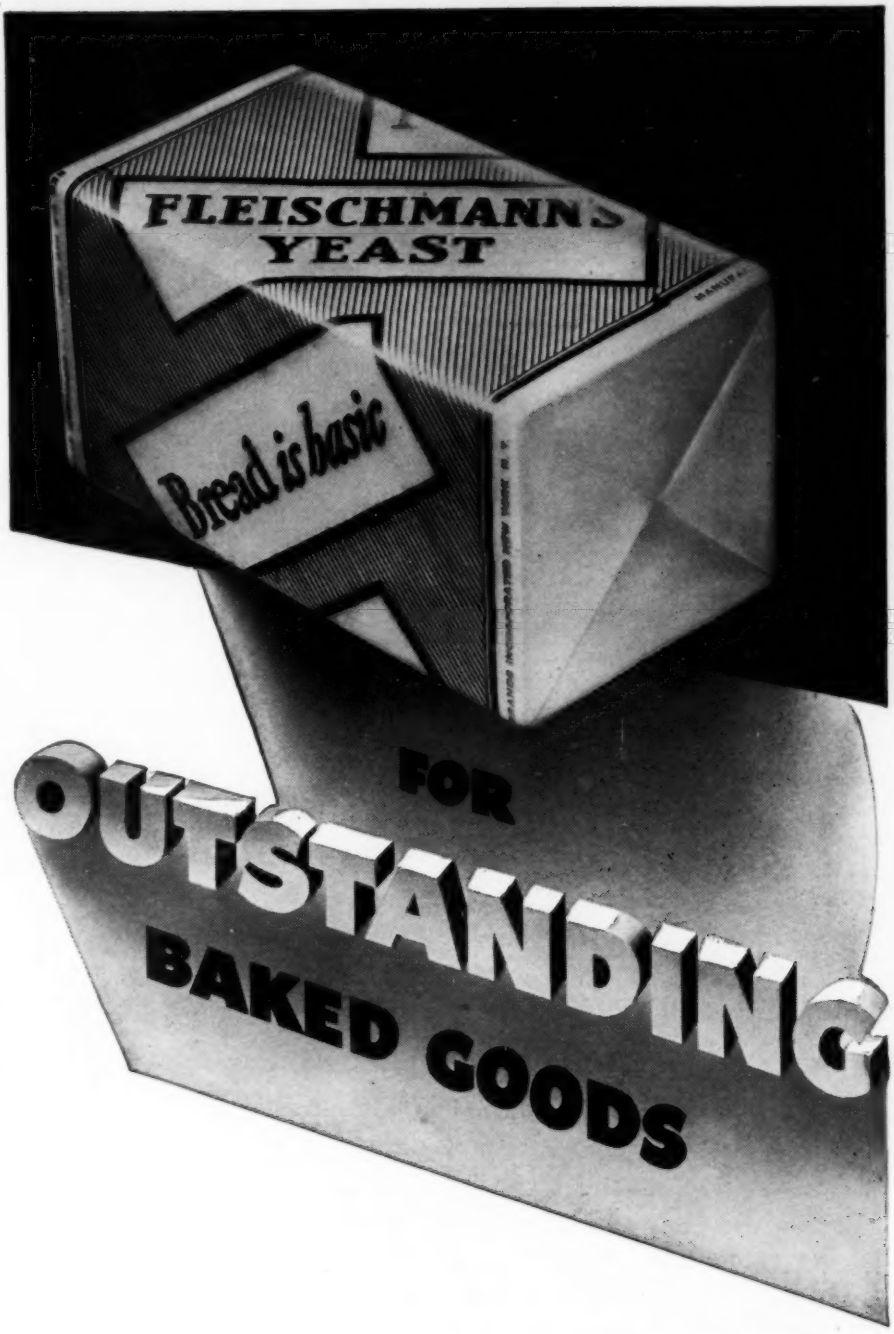

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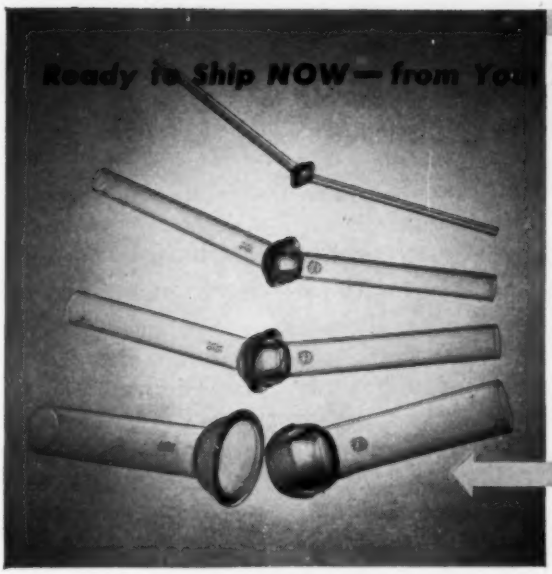

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CEREAL CHEMISTRY

VOL. XXIII JULY, 1946 No. 4

PHOTOMICROGRAPHIC STUDIES OF WHEAT STARCH. I. DEVELOPMENT OF THE STARCH GRANULES [1]

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(Received for publication March 4, 1946)

The starch granule has been the subject of an immense amount of microscopic study beginning with the work of Leeuwenhoeck in 1716 who concluded that the starch grain consisted of an outer insoluble, indigestible coat and an inner nutritive substance which disappeared when the granule was heated in water. Reichert (1913) gave a thorough review of the starch literature including that dealing with the origin and growth of the starch granule. He found the literature quite contradictory: "Very frequently the reports of one observer are not confirmed or are absolutely contradicted by those of others, even when the same method or reagent has been employed. . . ." (The starch granule), "as Poggendorff pointed out in 1836, was up to that time one of the most studied and least understood of all substances." Badenhuizen (1939) indicated that the situation was not much better in 1938. He suggested that if we review our present knowledge of the structure of the starch granule we are struck by the remarkable fact that we have actually arrived at a plane of knowledge equal to that of one hundred years ago.

According to Reichert (1913), Fritzsche (who apparently was the first to study the mechanism of the formation of the starch granule) concluded that all granules were formed by the deposition of the outer layers upon the inner. This "apposition" theory of the growth of the starch granule was accepted by other investigators until Walpers in 1851 proposed the view that growth was by "intussusception"; i.e., growth from the outside inward or by deposition within and between material already laid down. These opposing views led to considerable controversy which has not been entirely settled at the present time

[1] Published with the approval of the Director as paper No. 394, Journal Series, Nebraska Agricultural Experiment Station.

(Wieler, 1938) although it seems that the theory of growth by external apposition is quite generally accepted.

Investigations of the development of the wheat kernel have been extensive (Brenchley, 1909; Brenchley and Hall, 1909; Thatcher, 1913 and 1915; Eckerson, 1917; Jensen, 1918; Percival, 1921; Gordon, 1922; Miller, 1939; and many others). Since there is considerable similarity in the development of the grain of the different cereals, such investigations as those of Harlan (1920), Mottier (1921), Weatherwax (1930), Lampe (1931), and Randolph (1936) also throw considerable light on the development to be expected in wheat. However, none of this work was designed specifically to show the details of the growth and development of the starch granules.

Teller (1938) published photomicrographs of immature wheat and barley starch which supported his hypothesis that the amylases of grains and tubers were active in the formation of the starch granule, i.e., that growth of the starch granule was the antithesis of starch degradation by the enzymes. Bice, MacMasters, and Hilbert (1945) pointed out some of the differences between mature and immature wheat starch. Evans (1941) made a study of the development of corn starch and showed photomicrographs of granules at different stages of growth.

Wheat starch is composed of granules varying in diameter from 2 μ to 50 μ. It has been hypothesized that the ratio of small to large granules in a wheat has an effect on the baking properties of its flour (Buchanan and Naudain, 1923; and Stamberg, 1939), although Grewe and Bailey (1927) could find no relationship between baking properties and size of starch granules.

MacMasters and Hilbert (1944) state that in the preparation commercial wheat starch much of the small granule starch is lost from the better grades of starch and is recovered in the tailings. Consequently, in the manufacture of wheat starch the quantity of small granule starch is of industrial importance and a study of the origin and development of this starch may yield information of value to the wheat industry. It is the purpose of the present paper to present our observations concerning the development of wheat starch.

Materials and Methods

Selection of Kernels of Wheat of Approximate Known Age. For this study the procedure of making daily harvests of wheat kernels (Brenchley, 1909) was used, starting with the ovary before fertilization and continuing to the maturity of the grain. In order to have material of approximately known age, heads of hard winter wheat were dated at the first signs of pollination (extrusion of the first anthers); one kernel

was harvested from each of several of these heads each day. This procedure does not insure the exact age of the kernels since there may be considerable difference in the age of kernels on the same head (Brenchley, 1909); however, by harvesting kernels from several heads each day those obviously out of line in development could be discarded. Field-grown wheat was used during the first season of this study (1941), whereas both field and greenhouse samples were used during the succeeding seasons.[2]

Starch Preparations. Preliminary preparations consisted of starch squeezed directly from a kernel onto a microscope slide. In later studies the pericarp (plus the epidermis of the nucellus) and endosperm were separated permitting starch development to be followed in both the endosperm and pericarp. In those cases requiring washed starch, the starch was pressed from the tissues into water and washed by repeated suspension, settling or centrifuging, and decantation.

Cross sections of wheat kernels were cut on a freezing microtome using ethyl chloride as the freezing agent. Sections were cut both from fresh material and from material which had been fixed in formalin acetic alcohol.[3] The sections used for photomicrographic illustration were all fixed in formalin acetic alcohol.[4]

The photomicrographs were made under the direction of R. F. Morgan in the laboratories of the Department of Visual Education, using Bausch and Lomb GBVP photomicrographic equipment. A B & L mechanical-feed arc lamp and B & L polaroid disc polarizer and cap analyzer were used for studies involving birefringence. Such polaroid equipment gives results comparable to those obtained with a polarizing microscope (Young, 1944).

Movement of starch granules due to convection currents and to drying of the slides was prevented by sealing the sample under the cover glass. A narrow band of vaseline was placed around the edge, on one face, of the cover glass. After the sample was prepared on the slide, the cover glass was gently pressed into place using care to have the vaseline make a complete seal (McNair, 1930). Starch granules in samples thus sealed under the cover glass may be kept for weeks with little perceptible movement. No undue pressure should be applied to the cover glass as starch granules are quite easily damaged by pressure (Jones, 1940).

In the early stages of growth the starch granules were so small that they were subject to too much Brownian movement for observation or for photographing while in the original suspension. It was found

[2] Wheat used for these studies was grown primarily for other purposes by the Agronomy Department of the Nebraska Experiment Station.

[3] We are indebted to Dr. E. R. Walker of the Botany Department for her suggestions and aid in making these sections.

[4] Formalin acetic alcohol. Mixture of 7 *ml* acetic acid, 5 *ml* formalin, and 88 *ml* 50% ethanol.

that Brownian movement could be largely prevented, with no perceptible change in the character of the starch, by allowing the starch suspension to dry on the slide. On carefully adding water (or the particular solution desired) the granules were left lightly sealed to the glass; this permitted observation and photographing of granules less than 1 μ in diameter.

Staining. Most of the photomicrographs were made of unstained granules and of unstained sections of kernel tissue. However, in some cases it was necessary to use iodine as a stain in order to distinguish minute starch granules from other organized material in the cells of the kernel. The iodine concentration to be used was determined for each particular set of conditions by preliminary tests on similar material. Best results were obtained by using relatively large volumes of the minimum concentration of iodine which would stain the granules of the preparation under consideration.

Precise differential staining between different starch granules, or between parts of an individual granule, necessitated the use of relatively large volumes of exceedingly dilute iodine (0.0007 *N*). Inadequate volume of solution gives apparent differentials which may be quite misleading; this is due to the adsorption of iodine by the first granules contacted, thus leaving a lower concentration for succeeding granules. Too high concentrations of iodine stain all granules and all parts of granules so deeply that no distinctions may be made. Generally it is necessary to make preliminary observations of the effect of iodine concentration on the particular starchy material under study. Similar to the difference observed by Jones (1940) between damaged and undamaged granules, immature granules stain more readily and with lower concentrations of iodine than do mature granules.

Results and Discussion

Figure 1, showing the changes in the external appearance of the wheat kernel during its growth and development, is given as a reference for correlating the development of the starch with that of the kernel. Figure 1 *A* represents the unfertilized ovary, with *B*, *C*, *D*, *E*, *F*, and *G* following at two-day intervals; *H* is the mature dried kernel harvested about four weeks after pollination. At a stage corresponding to *F* the kernel has reached its maximum length but has attained less than half of its final dry weight (Brenchley and Hall, 1909; Percival, 1921; Miller, 1939).

Early Starch Development in the Ovary and Growing Kernel. Minute spherical starch granules, varying in diameter from about 1 μ to 5 μ, occur even in the unfertilized ovary (Fig. 2 *A*). That these are in reality starch granules may be shown by their characteristic appear-

ance under polarized light, although birefringence of the smallest granules is questionable (Fig. 2 *B*), and by their staining with iodine (Fig. 2 *C*). Badenhuizen (1939) and Lampe (1931) also observed young starch granules of narcissus and of corn which showed no birefringence. Bice, MacMasters, and Hilbert (1945) account for

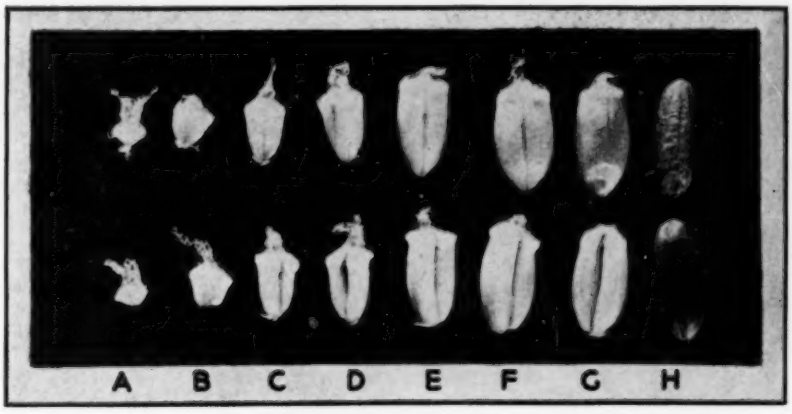

Fig. 1. Wheat kernels showing change in size and form during development (×2.5).
A. Unfertilized ovary.
B–G. Kernels approximately representative of development to be expected at 2-day intervals.
H. Mature dry kernel.

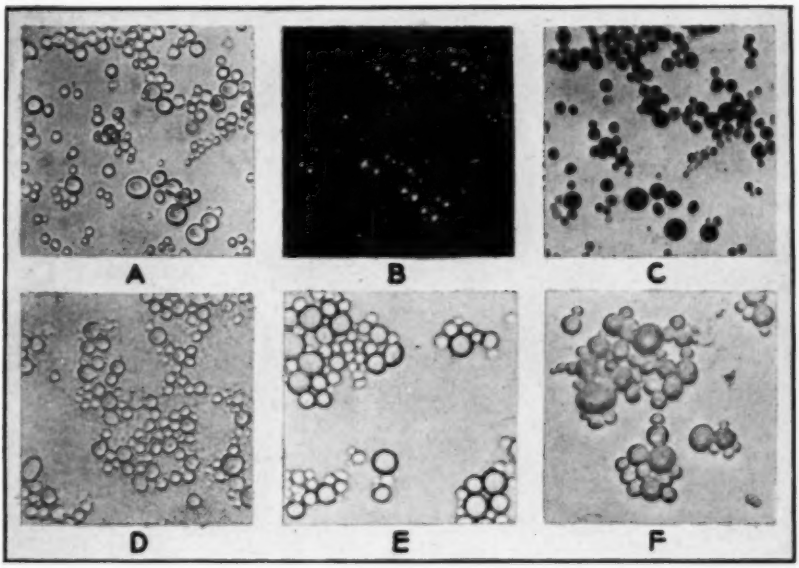

Fig. 2. Early starch development of the wheat kernel (×538).
A. Starch of the unfertilized ovary.
B. Field A between crossed polaroids.
C. Field A stained with iodine.
D. Starch from the entire kernel harvested the 2nd day after pollination.
E. Harvested 3rd day.
F. Harvested 4th day.

these failures to observe birefringence by the supposition that the illumination used was inadequate to show birefringence in small granules; yet their own illustration, as published, does not show birefringence in the smaller granules. This indicates less distinct birefringence in these granules with possibly no birefringence in the smallest. The B & L carbon arc light used in the present research gave adequate lighting.

The starch granule grows with the development of the kernel, as is illustrated in Figure 2 *D*, *E*, and *F* representing the starch squeezed from the whole wheat kernel on the 2nd, 3rd, and 4th days after pollination.

Except for the increase in size, there was no readily apparent change in the character of the starch during the first four days; however, at about the 4th day after fertilization corresponding to approximately stage *C* (Fig. 1) in the growth of the kernel, another type of starch was

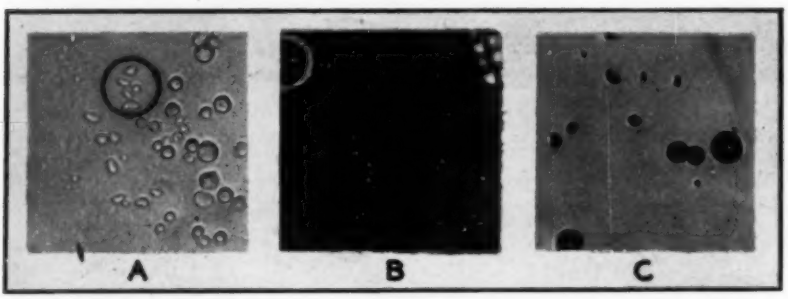

Fig. 3. First appearance of endosperm starch in the kernel (×564).
A. Harvested 4 days after pollination: circle encloses a group of endosperm granules.
B. Between crossed polaroids. Circle encloses the same group of initial endosperm granules in *A* and *B*. The field was shifted to bring two granules of normal birefringence into view.
C. Stained with iodine—original field lost in staining.

observed. The indistinct (due to small size and transparency) oblong or bean-shaped granules shown in Figure 3 *A* were characteristic of this second type of starch as it was first observed.

Figure 3 *B*, showing this starch under polarized light, indicates that any birefringence which it may have had was so slight as to be questionable; this may be due merely to the concentration of starch being too low [5] or to there being too little crystalline starch present at this stage to produce perceptible birefringence. As before noted, Badenhuizen (1939) and Lampe (1931) also observed young starch granules which showed no birefringence. Even though this starch does not show birefringence, it stains with iodine (Fig. 3 *C*). The original field of

[5] That the quantity of starch (thickness of the layer of starch) through which the polarized light passes may have considerable effect on the apparent birefringence of starch granules may be noted if the birefringence of overlapping immature granules is observed; the overlapping region shows more distinct birefringence than those regions not overlapping.

starch shown in Figure 3 *A* and *B* was lost in the manipulation necessary for staining, but both types of starch are evident in the field shown.

The second type of starch develops rapidly; in one day's time, the starch of the wheat kernel changes from one predominately spherical in nature to one of a mixture of various shapes. Figure 4 shows

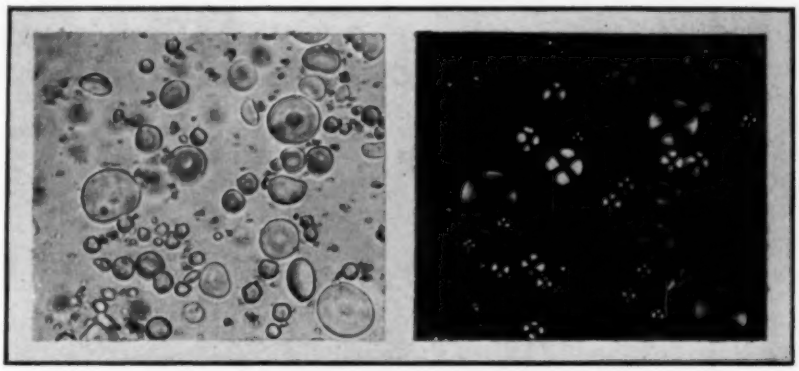

Fig. 4. Starch from a kernel 5 days after pollination (×538).
Right—between crossed polaroids.

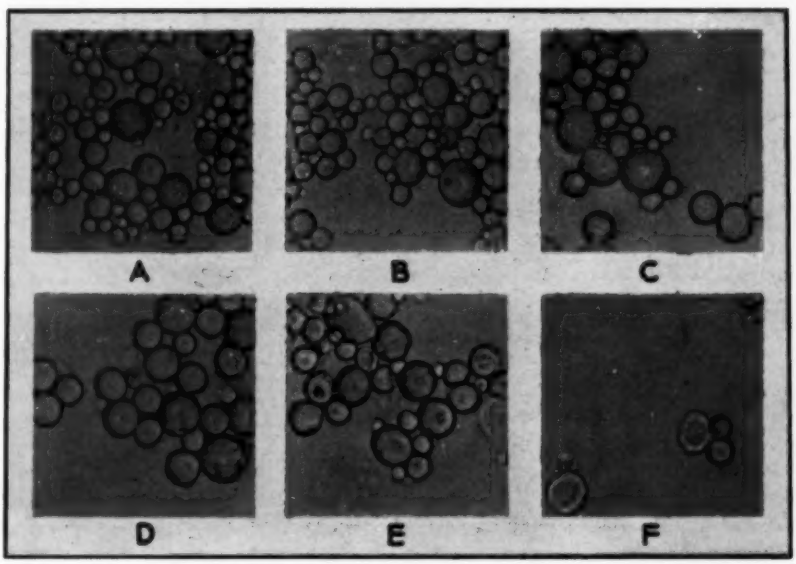

Fig. 5. Development of pericarp starch granules (continued from Figure 2) (×564).
A. 5th day—evidence of enzyme action at the hilum of the larger granules and some pitting of the granule surface.
B. 7th day.
C. 9th day.
D. 12th day—marked pitting of the surface of granules.
E. 14th day.
F. 16th day—the few granules left were largely digested.

starch as it appeared on the 5th day after pollination. At this stage the original spherical type starch tentatively may be distinguished from the new by its greater density, more definite birefringence, and different type of hilum.

Brenchley (1909) stated that "in all the younger stages there is an accumulation of starch in the pericarp." Jensen (1918) also stated that starch makes its appearance in the pericarp tissue long before any vestige of it is present in the endosperm. According to the literature (Brenchley, 1909; Miller, 1939; Percival, 1921; and others) starch storage in the *endosperm* starts after the tissue of the kernel has been completely formed; i.e., 10 to 14 days after fertilization of the flower

Fig. 6. Cross section of wheat kernel 3 days after pollination (×30).
P. Pericarp.
E. Free nucleate endosperm.
The dark border around the endosperm is composed of the ovular integuments and the nucellar wall (See details in Figure 7.)
Dotted lines enclose area approximately corresponding to that in Figure 7.

(or stage *F* of Fig. 1). This would indicate that both types of starch granule originated in the pericarp. However, when the pericarp and endosperm from kernels at various stages of development were separated and the starch from each was studied, it was found that the starch from the pericarp was all of the spherical type and that the endosperm contained starch as early as the 4th day after pollination.

The development of the pericarp starch from the 5th to the 14th day is shown in Figure 5. (This is a continuation of development

shown in Figure 2.) As stated by Brenchley (1909), the pericarp starch gradually decreases in quantity with time. Evidence of enzymatic digestion of the pericarp starch granules began to be noticeable by about the 4th or 5th day (Figs. 2 *F* and 5 *A*). In many of the granules digestion appeared to start at the hilum with the innermost portions of the granule disintegrating before the outer layers (Fig. 5 *C* and *F*), indicating that the first starch deposited (in the interior of the granule) was less resistant to amylase action than the outer, and younger, layers. It should be emphasized, however, that the enzyme

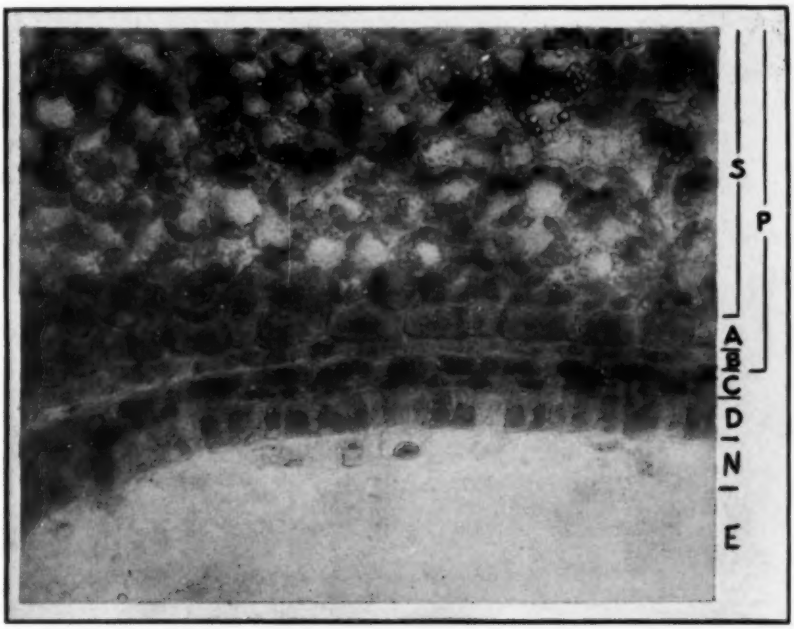

Fig. 7. Portion of a cross section of a wheat kernel 3 days after pollination (×164).
P. Pericarp with starch cells (*S*), cross layer (*A*), and inner epidermis (*B*).
C. Integuments of the ovule.
D. Epidermis of the nucellus.
E. Endosperm space from which the free nuclei were lost in cutting the section.
N. Remains of the thin-walled nucellar layer.

had digested channels through the outer layers of the granule before it had access to the interior; in some cases the surface became noticeably pitted before the disintegration of the interior (Fig. 5 *D* and E). Apparently the differences in resistance to amylase action between the inner and outer layers varied from granule to granule. By the 16th day, about two to four days after the kernel had reached maximum length, the pericarp starch had practically disappeared (Fig. 5 *F*).

Early Development of the Endosperm. To determine the time and place of origin of the two types of starch which were obtained from the

whole kernel on the 5th day of its development (Fig. 4), kernels of wheat representing the early growing period were sectioned. Figure 6, a cross section of a wheat kernel three days after pollination, shows the relation of the pericarp to endosperm at a stage of kernel development when only pericarp starch was present. The greater portion of the kernel consisted of pericarp which was largely composed of starch cells. The endosperm was small and contained no starch. Weatherwax (1930) describes the endosperm at this stage as a "multinucleate

Fig. 8. Cross section of a wheat kernel 4 days after flowering (×30).
P. Pericarp.
E. Endosperm.
A. Area corresponding approximately to that shown in Figure 9.

cell" which has developed from the definitive nucleus by repeated division; i.e., the endosperm consisted of a small mass of protoplasm containing many nuclei with no cell walls separating the nuclei.

The division of the endosperm into cells, i.e., the formation of cell walls between the nuclei of the endosperm, begins at the periphery of the "multinucleate cell" (Brenchley, 1909, and Percival, 1921). Figure 7 shows a portion of a section, thinner than that shown in Figure 6 and with higher magnification. The free nucleate endosperm was lost in cutting the thin section.

Figure 8 shows the relative development of the pericarp and endosperm in a cross section of a wheat kernel harvested four days after pollination (corresponding to stage *C* of Figure 1). A section of the endosperm before and after staining with iodine (corresponding to the area designated by *A* in Figure 8) is shown in Figure 9. The division of the endosperm into cells was exceedingly rapid; in this case seemingly it was complete in about one day's time (Brenchley, 1909, estimated the time as approximately two days).

Endosperm Starch. Even at this early stage, apparently as soon as the division of the endosperm into cells was complete, the deposition of starch had begun in the inner endosperm cells. The starch granules

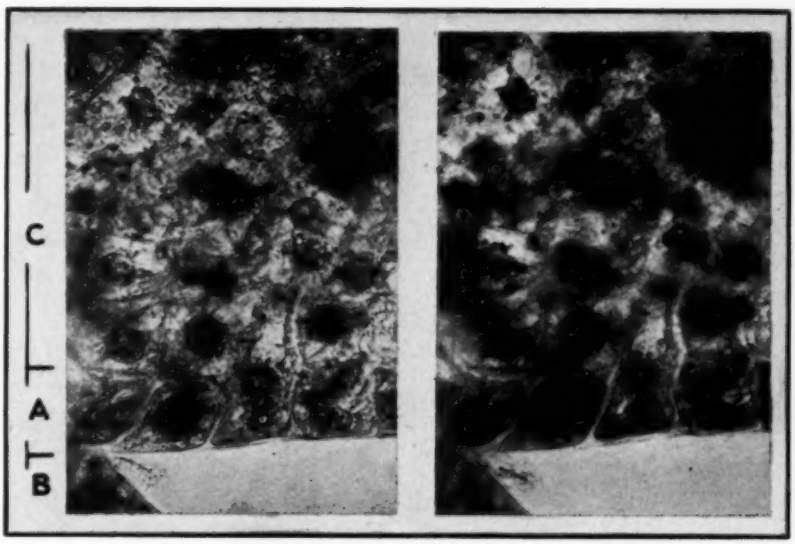

Fig. 9. Section of endosperm 4 days after flowering (×312). Right—same section stained with iodine.
A. Aleurone layer—peripheral layer of endosperm cells.
B. Epidermis of the nucellus.
C. Endosperm with the inner cells showing initial starch granules.

as they were just beginning to form were difficult to distinguish from other structures of the cell until stained with iodine. Figure 10 shows a starch cell, stained with iodine, which was isolated from the endosperm at this stage of development. The stained starch granules of this cell are similar to those shown in Figure 3. Apparently endosperm starch starts to develop much earlier than has been supposed (Brenchley, 1909; Miller, 1939; Percival, 1921).

The peripheral layer of cells of the endosperm, which was the first to develop cell walls (Brenchley, 1909; Percival, 1921; and Gordon, 1922), did not contain starch (Fig. 9).

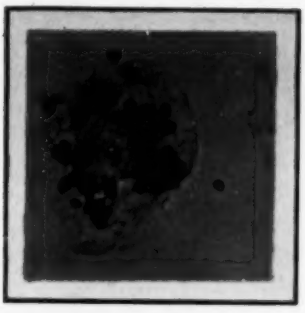

Fig. 10. Endosperm starch cell with initial granules, stained with iodine (×600)

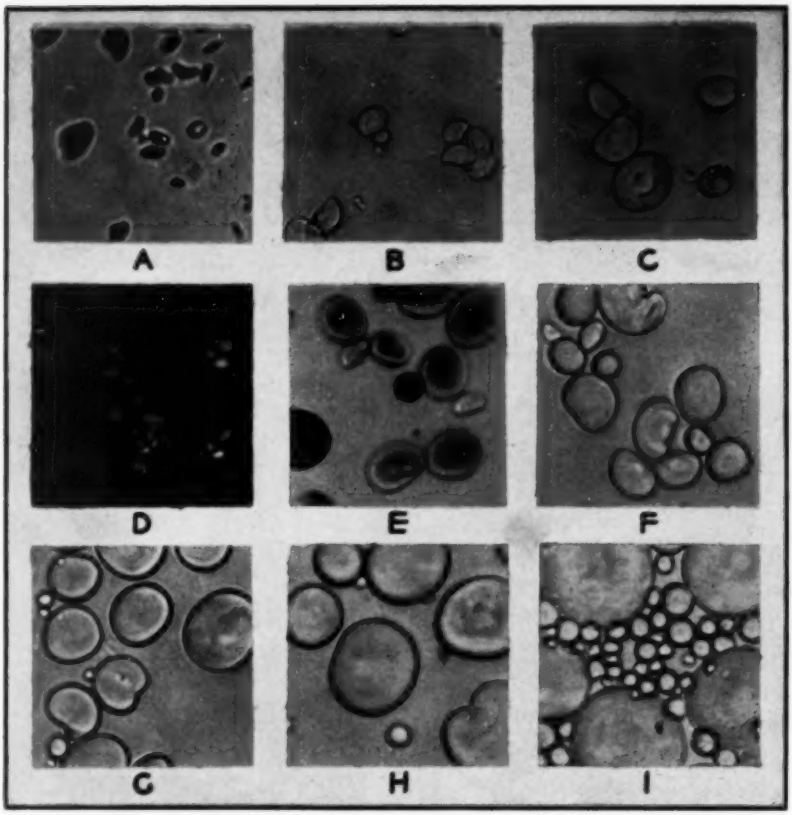

Fig. 11. Development of endosperm starch granules (*A:* ×1200, *B–I:* ×564).
A. Initial granules—5th day after pollination. Stained with iodine (×1200).
B. 7th day.
C. 9th day.
D. Same field as *C* between crossed polaroids.
E. 9th day stained with iodine.
F. 11th day.
G. 13th day—first appearance of spherical granules.
H. 15th day.
I. Granules from mature dry wheat.

The first endosperm starch that could be isolated was quite transparent and did not photograph satisfactorily. Stained with iodine it appeared as shown in Figure 11 *A* (compare with Fig. 10). The development with time is shown in the succeeding photomicrographs of Figure 11. It is interesting to speculate on the method of growth of

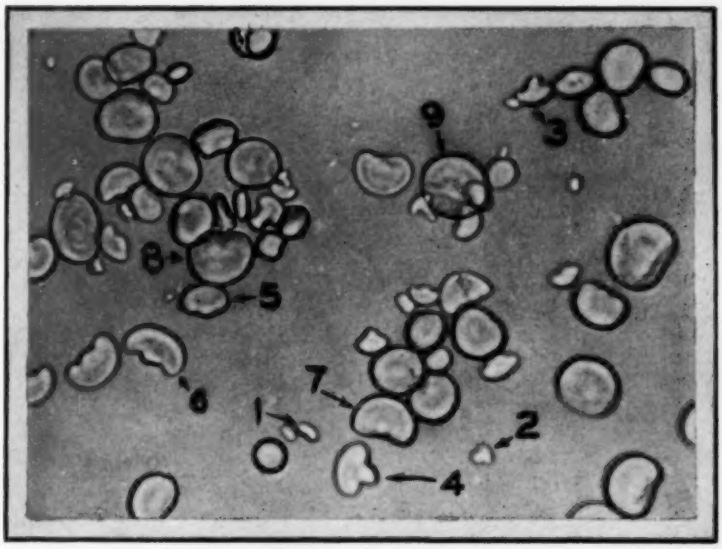

Fig. 12. Various stages in the development of endosperm starch granules—as found in a kernel harvested 12 days after pollination (×600).
Numbers (1,2,3,etc.) indicate possible order in stage of development.

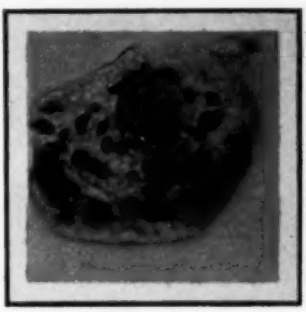

Fig. 13. Endosperm cell containing starch granules which probably are only 1 or 2 days old. Stained with iodine (×600).

the granules. Perhaps, judging from the shapes of the granules observed during the early stages of growth, the individual granule develops through a series of forms as indicated by the numbers *1*, *2*, *3*, etc., in Figure 12. Beginning with a sphere which serves as a "nu-

cleus" or hilum [6] on which more starch deposits, the form of the developing granule would be dependent on whether the deposition starts at one point with growth around the hilum from that point or whether the deposition starts at two or more points. Figure 13 shows the various shapes of the developing starch granule as they may be observed in the starch cell. In so far as one can judge, this cell was probably only a day older than that shown in Figure 10.

In general the growth of the granule is much more rapid in one plane, thus forming (when the "nucleus" is completely enveloped) a disc with a prominent bulge in its middle. As the granule grows and approaches maturity, it becomes thicker and more dense with the "nucleus" becoming less prominent.

That the middle portion of the granule is not a "spherical space" or cavity, as is suggested by the "growth by intussusception" theory, may be seen under the microscope by allowing evaporation to take

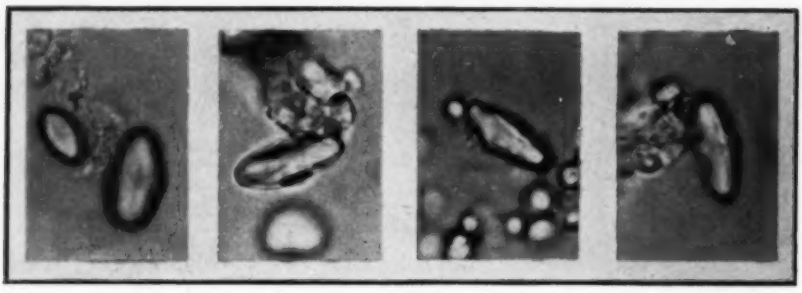

Fig. 14. Edgewise views of immature endosperm starch granules showing the bulging "nucleus" in profile.

place at one edge of the cover glass; as the immature granules float across the field under the microscope some of them roll over and as they turn edgeways the prominent bulge in the middle may be clearly seen. Figure 14 shows some immature granules as they appear when viewed from the edge.

The bulge in the middle of the granule gives a more distinct birefringence and stains more deeply with iodine than does the surrounding starch (Fig. 11 *D* and *E*). Denniston (1907) suggests that the difference in staining between the inner and outer portions of the starch granule indicate that the outer portion is a transition layer. According to the above observations this seems to be a logical explanation. However, the fact that many waxy starch granules (as found in the waxy sorghums, corn, and barley) have a center which stains blue with iodine (Meyer, 1886, and Lampe, 1931) indicates the

[6] Perhaps this beginning sphere should be called the hilum of the developing granule since the hilum "is said to be the organic center or nucleus around which the granule has grown" (Trubell, 1944).

possibility of there being considerable difference in the type of starch laid down in a granule at different times and under different conditions.

The immature granules are transparent and flexible, stain readily with dilute iodine, and have indistinct birefringence, which suggest a highly hydrated starch; i.e., a semiliquid starch with perhaps relatively few bonds between molecules. With growth the starch granules become less flexible, more dense, and thicker, and their birefringence becomes more distinct. Perhaps these changes in the granule may be explained by a decrease in hydration with a greater number of bonds formed between molecules (the bonds formerly holding water of hydration being available to form bonds between molecules). Deposition of more starch, either by apposition or by intussusception, in such a case would account for the thickening of the granule and also may be a factor in producing more definite birefringence.

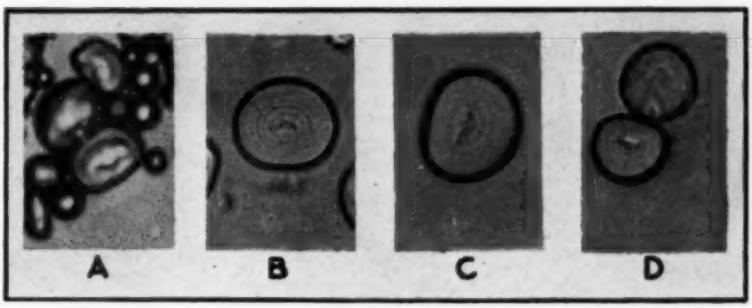

Fig. 15. Endosperm starch granules with bean-shaped hila.
A. Immature granule (×660).
B–D. Partially gelatinized mature granules (×440).

Some of the immature forms of the developing granule may be observed serving as "nuclei" of older granules. In Figure 15, *A* shows an immature granule with a bean-shaped "nucleus." The "nucleus" of the mature wheat granule is not ordinarily visible under the microscope; however, by careful partial gelatinization it may be differentiated from the surrounding structure. Figure 15 *B*, *C*, *D* shows mature granules gelatinized in water at 55° with the bean-shaped "nuclei" plainly visible.

The Spherical Type of Endosperm Granule. Most mature starches prepared from pure material are composed of granules varying widely in size. Usually, however, all of the granules from a particular material are of one definite type. Wheat, barley, and rye starches are peculiar in that they contain two distinct types of granules: Small *spherical* granules and larger lenticular granules. By manipulating the cover glass, or by allowing evaporation from the edge of the cover

glass, so as to cause a rolling movement of the granules of mature wheat starch, the spherical nature of the small granules as contrasted with the well-known lenticular character of the large granules is readily observed.

At about the time that the kernel attained its maximum length (corresponding to stage *G* of Figure 1 which represents the development at about 12 to 14 days after pollination) a few small granules began to appear among the lenticular endosperm granules (Fig. 11 *G*). Up to this time the granule population of a cell was relatively uniform in size. By the time the kernel had reached maturity, these small granules

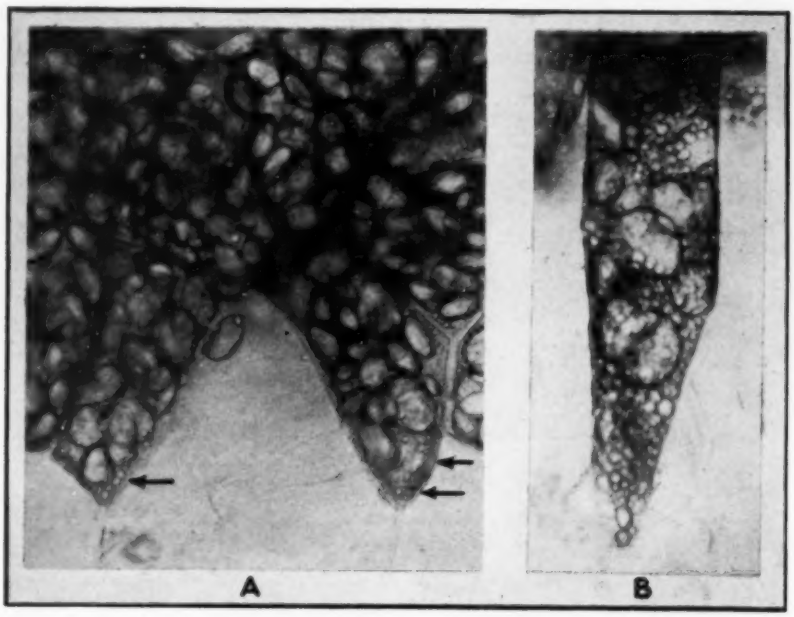

Fig. 16. Endosperm cells showing development of spherical granules (×373).
A. Showing the beginning deposition of spherical granules—arrows.
B. Mature cell with available space packed with spherical granules.

were present in large numbers and could be seen to be spherical. They attained a maximum diameter of about 10 μ as compared to about 35 μ for the lenticular granules (Fig. 11 *I*).

Figure 16 illustrates the development of the spherical type of granule in the endosperm starch cells. The cells in *A* show the beginning deposition of spherical granules; the lenticular granules had attained complete circular form. Cell *B* was from a mature kernel of wheat showing the small spherical granules completely filling all available space. As may be seen by comparing the size of the starch granules of the cells shown in *A* with those in *B*, the lenticular starch con-

tinued to grow during the period of deposition and growth of the spherical granules.

Mature lenticular wheat starch granules quite generally show surface markings similar to those on the granules of Figure 11 *I*. On careful examination under the microscope these appear to be indentations in the surface. A comparison of these markings with the appearance of the small granules overlying the large granules in the mature starch cell (Fig. 16 *B*) suggests that the packing of the small granules into all available space in the cell may produce indentations on the large granules which become permanent during the late growth and drying of the kernel.

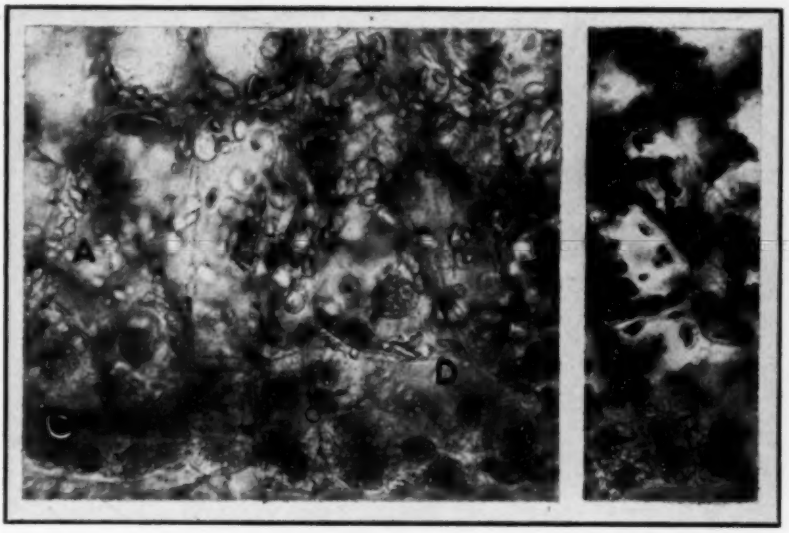

Fig. 17. Section of the endosperm 6 days after flowering (×300).
A. Cell containing bean-shaped granules.
B. Aleurone layer.
C. Newly formed starch cells before starch deposition had started. Right—similar section stained with iodine.

The Function of the Aleurone.[7] During the early development of the wheat kernel, the lenticular type of starch as pressed from an entire endosperm consisted of a mixture of granules in various stages of development. However, on examining cross sections cut from the middle and both ends of a kernel, it was evident that the granules of any particular cell were all quite uniformly developed (until the appearance of the spherical granules), whereas there was a gradation in development of the starch cells from the periphery inward.

[7] In the normal course of kernel development the outer or peripheral layer of cells of the endosperm becomes the aleurone of the mature kernel. During the early stages of development this peripheral layer does not have the characteristics generally associated with mature aleurone. In this paper, the term aleurone is used to designate the peripheral layer of endosperm cells at all stages of development.

Figure 17 shows a portion of the cross section of the endosperm of a kernel of wheat harvested six days after pollination. The starch granules in the interior of the endosperm had developed to about the stage of the larger granules shown in Figure 11 *C;* the cell *A* contained granules at the "bean-shaped" stage, whereas the cells nearer the periphery contained granules in still earlier stages of development (*D*). Staining a similar section with iodine (Fig. 17—right) shows that the aleurone (*B*) and the next layer within (*C*) contained no starch granules (a few granules may be carried into this area by the microtome in sectioning). The apparent gradation of starch cell development from the periphery to the middle of the endosperm indicates a gradation in age of cells. It is significant that in the cross sections of the

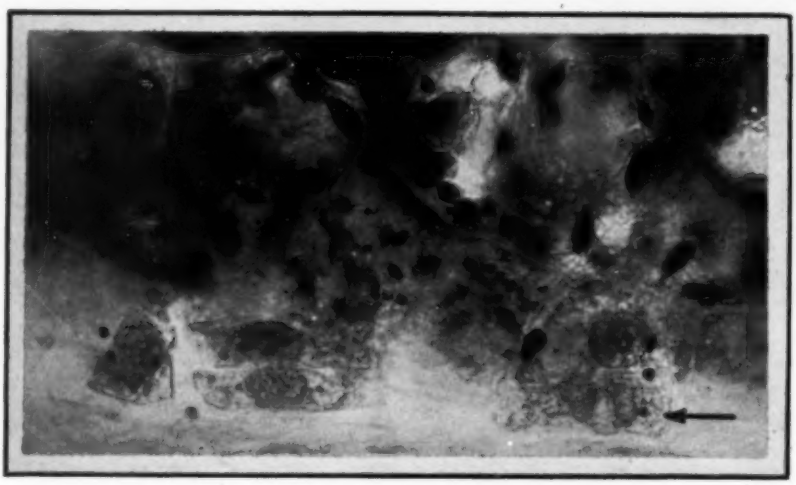

Fig. 18. Section of endosperm stained with iodine showing radial cell division in an aleurone cell (×546).
Arrow points to cell in the telophase of division. The two daughter nuclei are clearly visible.

immature kernel (Figs. 9, 17, 19, and 20) the individual cells in the first row of cells inward from the aleurone line up with the aleurone cells—a young starch cell corresponding to one or two aleurone cells.

Gordon (1922) proposed the theory that the aleurone layer during the early growth of the kernel is a meristematic cambium tissue which produces new endosperm cells. Mottier (1921) expressed a somewhat similar view concerning the peripheral region of some other cereals, although he thought that the most active meristematic cells lay just inside the aleurone. The photomicrographs of cross sections of the immature wheat kernel shown in the present paper lend support to the theory that the aleurone during the growth of the grain is a meristematic tissue and that its function is the production of endosperm cells.

Apparently the aleurone cells divide both tangentially, producing daughter starch cells, and radially, producing new aleurone cells. Two aleurone cells corresponding to one starch cell would indicate that a radial division took place after the last tangential division (Fig. 19 *B*). Gordon (1922) showed tangential division in her illustrations. Figure 18 shows radial mitotic division taking place in an aleurone cell. This section was stained with iodine and accordingly also shows the range in starch granule development from the aleurone inward.

In the early development of the grain, the only visible evidence of differentiation of the peripheral layer of the endosperm to form the

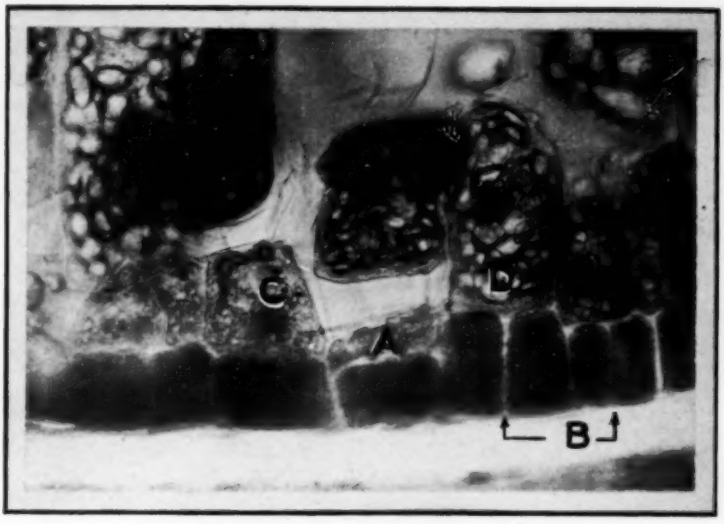

Fig. 19. Section of endosperm 12 days after flowering (×330). (Some cells displaced due to thin sectioning.)

A. Young starch cell arising from a tangential division.
B. Pairs of daughter cells produced by radial divisions in the aleurone layer. Each pair corresponds to a single starch cell.
A, *C*, and *D* illustrate the progressive steps in the differentiation of a starch cell.

aleurone was the absence of starch (Figs. 9 and 17). As the grain developed, the differentiation of this layer became more evident; it became more dense in appearance due to the deposition of "aleurone granules" (Gordon, 1922). Figure 19 shows a section of a kernel harvested 12 days after pollination. This would correspond to a stage similar to that shown in Figure 1 *E*. By this time the aleurone layer was definitely, visibly differentiated. Most of the first layer of cells just inside the aleurone showed starch granules in the early stages of development, though varying somewhat in age from cell *C* to *D;* this second layer was more developed (or older) than the corresponding layer in the earlier stages of growth (Figs. 9 and 17), indicating that the

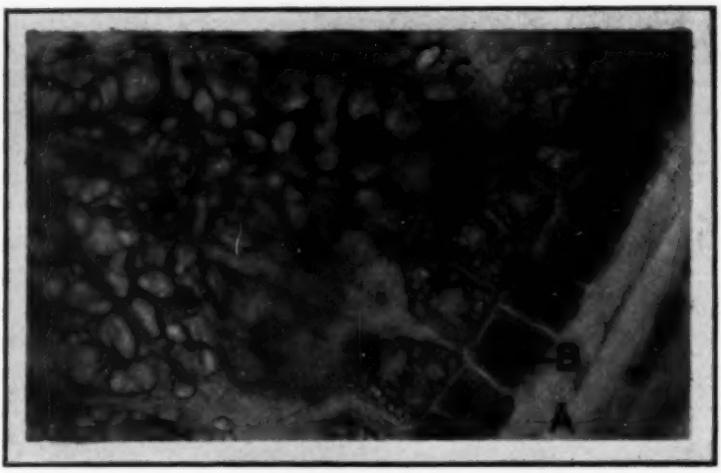

Fig. 20. Section of endosperm 14 days after flowering (×330).

A. Remains of the nucellus.
B. Aleurone.
C. Cell showing beginning deposition of spherical starch granules.

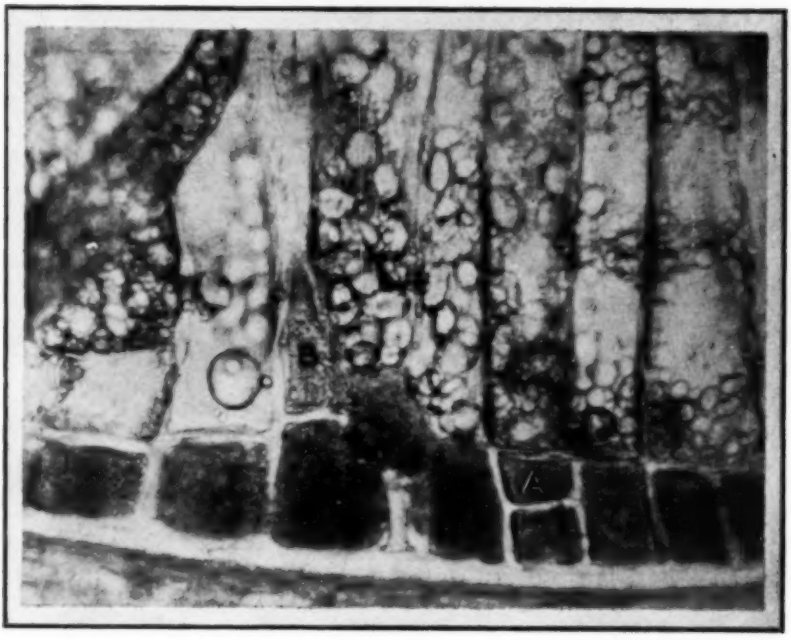

Fig. 21. Section of endosperm 16 days after flowering (×287).

A. Newly formed starch cell, not yet visibly differentiated from the mother aleurone cell.
B. Newly formed starch cell with starch granules beginning to form.

rate of division of the aleurone cells was decreasing with age. Evidence of recent division, however, was still found: a few cells had divided so recently (Fig. 19 *A*) that the inner cell still contained no starch but was similar in appearance to its mother cell.

Figure 20 shows a section of kernel harvested about 14 days after pollination. Apparently by this time aleurone cell division had slowed down considerably. Cell *C* contains a few newly formed granules of the spherical type of starch.

As may be seen in Figure 21, a section cut from a kernel harvested 16 days after pollination, there is still evidence of recent aleurone

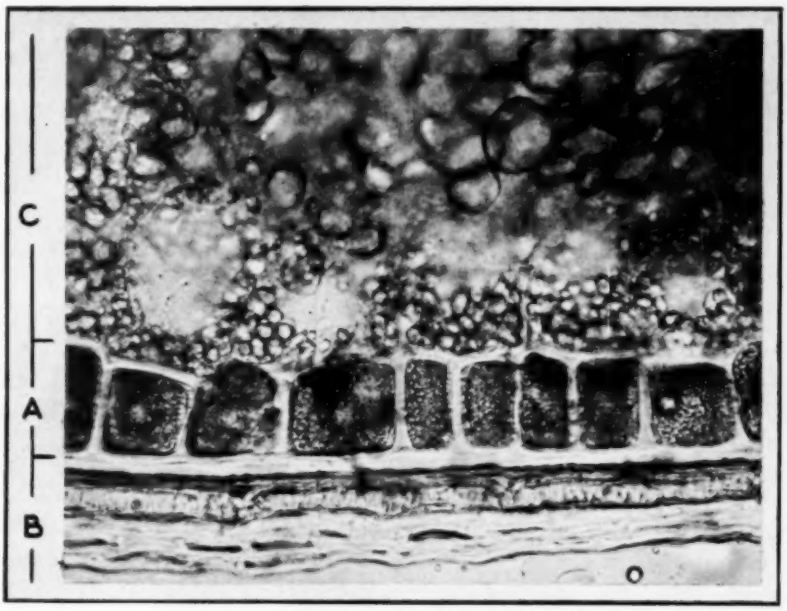

Fig. 22. Section of mature wheat kernel showing smaller granules in the layer of starch cells just within the aleurone (×287).
A. Aleurone.
B. Bran.
C. Starchy endosperm.

cell division at this late stage in the kernel development. Cell *A* was too young to show differentiation from its mother cell, whereas cell *B* had begun to develop starch granules.

Even sections cut from mature kernels of wheat show starch cells containing small starch granules just within the aleurone layer (Fig. 22). Apparently the aleurone continues to form new starch cells until so late in the growth of the kernel that the last-formed cells and the granules within the cells do not complete their development before the kernel matures; this seems to apply particularly to those cells at each side of the "furrow" or "crease" of the kernel.

Summary

The development of wheat starch granules in the maturing kernel was followed by means of photomicrographs.

Minute starch granules are present in the unfertilized wheat ovary.

Pericarp starch develops rapidly showing maximum diameter of granules by the 4th or 5th day. However, an enzyme is present in the pericarp which is capable of digesting starch granules; accordingly, the larger starch granules show indications of being attacked by the time they have reached maximum diameter. Pericarp starch practically disappears from the wheat kernel (due to enzymatic digestion) within a couple of days after the kernel attains its maximum length.

Minute endosperm starch granules may be found in the interior of the endosperm at about the time that cell walls form in this tissue, or approximately the 4th or 5th day after the flower has been fertilized. These granules develop into the large lenticular granules which are characteristic of wheat starch. Rye and barley starches also contain this type of granule.

Small spherical granules begin to develop in the cells containing lenticular granules at about the time the kernel attains full length. These granules completely fill the space between the lenticular granules.

Evidence is given supporting Gordon's (1922) contention that the peripheral layer of endosperm cells (the aleurone layer of the mature grain) is a meristematic tissue which produces new endosperm starch cells.

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THE PROTEOLYTIC ENZYME ACTIVITY OF MILLED FRACTIONS OF WHEAT AND THE EFFECT OF BROMATE IN FLOUR

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In a previous communication (1945) the authors considered certain properties of the proteolytic enzymes of milled fractions of wheat, and Howe (1946) showed that when the enzyme activity in germ extract was reduced drastically by hexylresorcinol or sodium fluoride, almost no effect on dough quality could be observed by farinograph studies. This finding was taken as evidence against the proteolytic theory of the effect of redox agents on dough quality.

The present investigation deals with the quantitative distribution of proteolytic activity among the milled fractions, and the effect on the activity of commercial levels of bromate in a straight grade flour. It was the purpose of the latter study to elucidate further the mechanism of bromate action with respect to the proteolytic theory.

Materials and Methods

All milling separations were obtained from the same mix made up entirely of 1945 crop spring wheat. The samples were stored in a refrigerator.

The measurements of proteolytic activity were carried out as follows: The whole wheat, bran, germ, and shorts were ground in a Wiley mill and the material passing a No. 20 sieve was used. A 2-g sample was weighed into a 200-ml Erlenmeyer flask and 10 ml of distilled water was added. The mixture was agitated until a uniform suspension was obtained and it was allowed to stand at room temperature for one hour. Ten ml of $M/7$ sodium acetate was added, followed by 5 ml of 0.75% cysteine hydrochloride and 5 ml of 6% casein, prepared as described previously by the authors (1945). With stirring, 1 N acetic acid was added to bring the pH to 5.0; this usually required 1 ml. Distilled water was finally added to bring the total volume to 35 ml, and four drops of chloroform were added to inhibit the growth of microorganisms. A separate flask with 35 ml of reaction mixture was used for each point on the activity-time curves shown in Figures 1 and 2. With any given reaction mixture one flask was emptied into a centrifuge tube as soon as it was made up, centrifuged for two

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minutes, at 2000 r.p.m. and 10 ml of the supernatant was titrated for amino groups by the acetone method previously employed (Howe and Glick, 1945). Other flasks were placed in a thermostat at 37°C for various periods up to six hours, after which the liquid was centrifuged and 10 ml of the supernatant was titrated as above. Control experiments were carried out by heating the mixture to boiling under a reflux condenser. The samples were placed in a thermostat for periods up to six hours, and the liquid centrifuged and titrated as

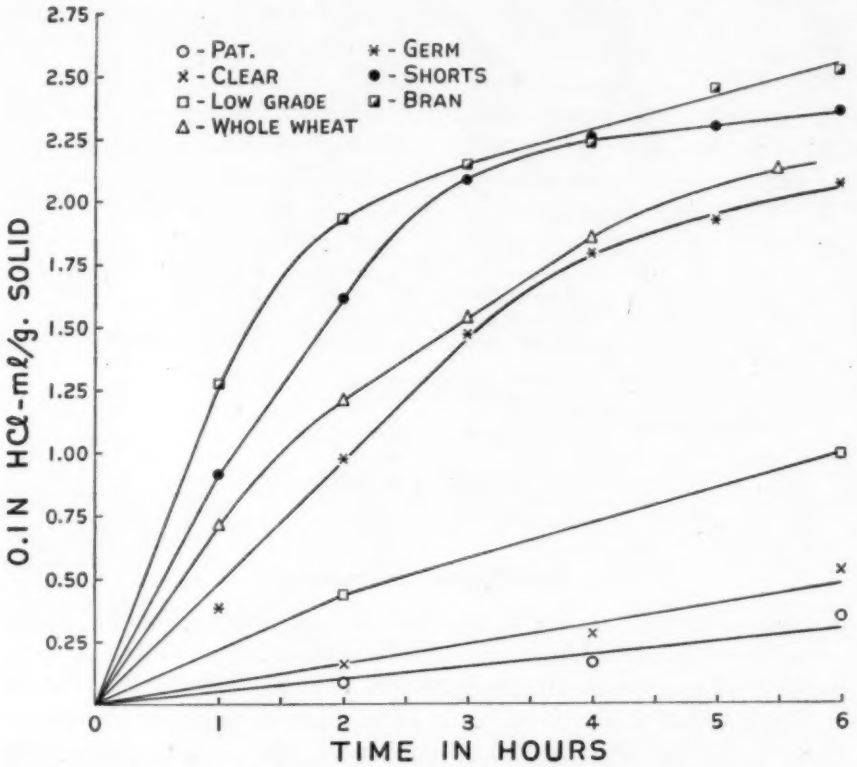

Fig. 1. Rate of proteolysis by milled fractions of wheat per gram of sample.

previously described. Nitrogen was determined on all of the fractions, and the results calculated to a 14.0% moisture basis.

The bromate experiments were set up in the same manner with and without the casein, but with no cysteine. In all cases the volume was made up to 35 ml with water. These experiments were all performed on a straight grade flour, and amounts of a 4 mg % solution of potassium bromate were added in place of some of the water to give flour-bromate ratios in a range (0.001–0.010% bromate in the flour) covering that used in commercial practice.

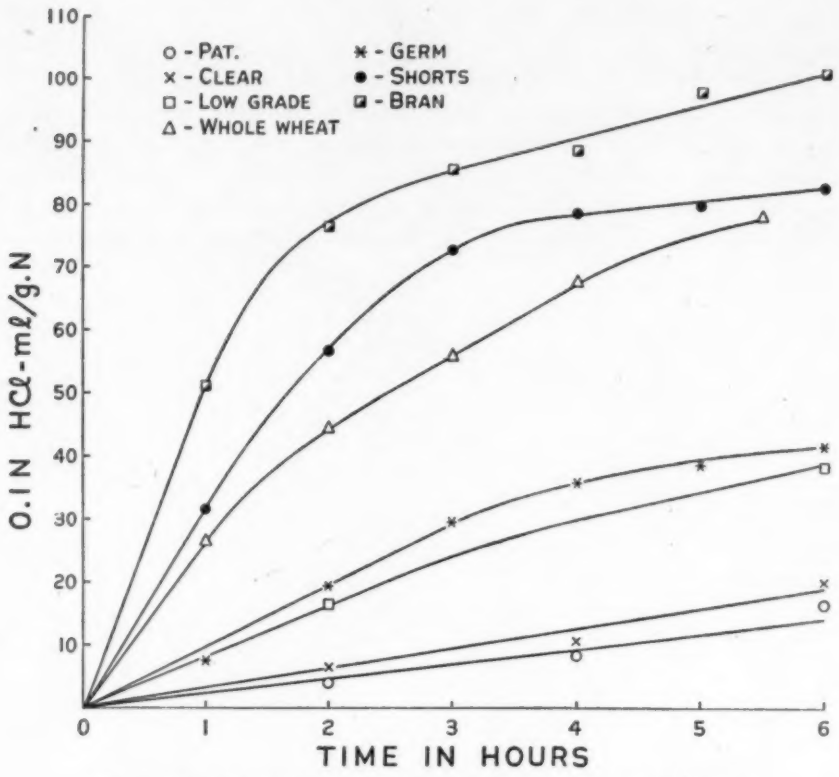

Fig. 2. Rate of proteolysis by milled fractions of wheat per gram of nitrogen.

Results and Discussion

From Figures 1 and 2 it is apparent that the order of the proteolytic activities of the milled fractions, based on either the amount of nitrogen in, or total weight of, sample is: patent < clear < low grade < germ < whole wheat < shorts < bran. The magnitude of the differences between the fractions is also approximately the same in both cases. The greatest deviation is found in the germ whose activity is closer to that of whole wheat when referred to unit weight of total sample; while it is closer to that of low grade when referred to unit weight of nitrogen in the sample. Balls and Hale (1936) reported that proteolysis followed the order: whole wheat < bran < germ. They did not indicate whether the fractions were obtained from the same wheat mix; and unless they were, the results could not be properly compared..

The effect of commercial levels of bromate on the protease activity of straight flour is shown in Table I. Practically no effect was exerted by the bromate on the six-hour titration, and the presence of casein did not measurably increase the value. After 24 hours the effect of

the added casein was clearly apparent, but the bromate exhibited no demonstrable inhibition of the activity at the concentrations employed; in fact, a tendency toward activation may be noted at the lower bromate concentrations. Of course, an oxidizing agent such as bromate is known to inhibit the protease action at higher concentrations, but apparently the levels used in commercial practice are well below the range at which inhibition occurs. This finding is in agreement with the conclusions of Read and Haas (1937), and it serves to weaken even more the highly tenuous foundation of the proteolytic theory of the action of bromate on dough properties. As pointed out by many investigators in the past, the proteolysis of wheat and its products is extremely weak. Our experiments again demonstrate that only a negligible percentage of protein can be hydrolyzed in periods far in excess of those required to show an effect on dough property, even under conditions for the maximum enzyme action.

TABLE I

EFFECT OF POTASSIUM BROMATE ON THE PROTEOLYTIC ACTIVITY OF STRAIGHT GRADE FLOUR

	Reaction time	Proteolytic activity [1]			
		Percent potassium bromate in flour			
		0.000	0.001	0.005	0.010
	hours	*ml*	*ml*	*ml*	*ml*
No casein	6	0.10	0.15	0.12	0.12
With casein	6	0.10	0.14	0.15	0.10
No casein	24	0.15	0.25	0.20	0.15
With casein	24	0.34	0.38	0.39	0.28

[1] Expressed as increase in 0.1 *N* HCl required to titrate the amino groups.

It is interesting to consider in this connection the defense offered by Jørgensen (1945), pages 325–336, for the proteolytic theory in the light of the facts revealed by Read and Haas (1937). Jørgensen readily admits the soundness of the experimental evidence presented by Read and Haas to the effect that no depression in nitrogen solubility is apparent in extraction experiments when bromate is added to flour at commercial levels, and only at higher levels can the depression be demonstrated. However, it is Jørgensen's claim that this finding is not in opposition to the proteolytic theory because, according to him, more bromate would be required since the ratio of water to flour was greater in the experiments of Read and Haas than is found in dough, and the presence of yeast in dough adds protease activators which were not present in the reaction mixtures of Read and Haas. It is sur-

prising that Jørgensen should choose to circumvent the serious obstacle placed in the way of the proteolytic theory by advancing arguments which support so clearly the point of view he wishes to refute. For, certainly, dispersing flour in water cannot hinder the interaction of bromate with the reducing substances in the flour; rather it would aid in the process. The critical ratio is that of bromate to the flour which contains the reacting components. And as for the point that reducing materials from yeast were not present in the experiments of Read and Haas, one can be sure that the addition of such reducing substances would only lead to a greater bromate requirement in dough and not a lesser one, as claimed by Jørgensen.

Summary

The relative proteolytic enzyme activities of milled fractions of wheat were determined quantitatively and found to fall in the order: patent < clear < low grade < germ < whole wheat < shorts < bran.

The proteolytic activity of a straight flour was not inhibited by the presence of potassium bromate in concentrations covering the range of those employed commercially. This is taken as additional evidence against the proteolytic theory of bromate action in dough. It was found that flour protease, fully activated by additional cysteine, can hydrolyze only a negligible percentage of the protein in six hours.

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OPEN TROUGH AND CABINET FERMENTATION OF BREAD SPONGES

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In the usual bakery practice, sponges are fermented in open troughs, preferably in a fermentation room controlled for temperature and humidity. If fermentation is conducted in cabinets, the air space over the sponge is minimized and the process is called cabinet fermentation. Cabinets may be built of a variety of materials from plywood to steel and should be 10 to 12 inches above the maximum height of the sponge. Cabinets are usually provided with doors to allow the entrance of the troughs.

Several bakeries have installed cabinets and have found superior results. Other shops have tried cabinet fermentation and have seen no advantage in the method. Hence, the subject has elicited much interest and heated discussion among bakers.

Johnson (1945) described experiments on small doughs made in the laboratory using different gas mixtures above the fermenting sponge. He found that the rate of expansion and height of the sponge were not affected by changing the concentration of carbon dioxide, oxygen, or nitrogen. However, an atmosphere varying between 15.5 to 25.0% carbon dioxide, 14.0 to 15.5% oxygen, and 61.0 to 69.0% nitrogen for a three-hour sponge produced better bread than the control sponge fermented in air of normal composition. Poor handling doughs resulted from the sponges fermented in high concentrations of oxygen and nitrogen.

Schoonover, Freilich, and Redfern (1946) studied cabinet fermentation on commercial size doughs and made careful measurements of temperature throughout the sponge. The humidity used in the dough room was 96%, which is unusually high. They reported that cabinet fermentation is of no value under controlled conditions in which the room temperature is equal to or slightly higher than the temperature at which the sponge comes from the mixer. However, when the dough room temperature is much cooler than the starting temperature of the sponge, cabinet fermentation showed definite advantages over open trough fermentation. They concluded that the cabinet method has no advantages in shops having accurate temperature and humidity control.

The authors have been interested in the subject of cabinet fermentation since its inception. Numerous comparisons of cabinet and

open sponges have been made, but the following experiments should serve to illustrate our results.

Materials and Methods

A spring wheat patent flour of 0.39% ash, 12.50% protein, and 500 mm gassing power (sixth hour) was employed in this work. The same brand of yeast at the same level was used throughout these tests.

A commercial-type sponge and dough procedure was followed using the formula:

	Sponge *lbs ozs*	Dough *lbs ozs*	Total-Flour Basis *%*
Flour	39	26	100.0
Water	23	21	67.7
Yeast	1 5	—	2.0
Yeast food	2	—	0.2
Shortening	1 15	—	3.0
Salt	——	1 5	2.0
Sugar	——	3 4	5.0
Nonfat milk solids	——	2 7	3.75

All standard bakery equipment was used; a water-jacketed, high-speed mixer (65 rpm), troughs 45 inches long, 26 inches wide, and 21 inches deep, divider, rounder, overhead proofer, and molder. The cabinet was built of plywood and fitted securely over the trough. There was a small glass window on top of the cabinet so that the sponges could be readily observed. Sponges at maximum height were 10 to 12 inches below the roof of the cabinet. Sponges were mixed for 5 minutes and taken from the mixer at 78°F (25.6°C). The room temperature was kept uniformly at 80°F (26.7°C), and the humidity maintained quite constant.

Samples for gas analysis were taken approximately one inch above the sponge. A piece of 2 mm glass tubing, fitted at one end with a 2-inch length of rubber tubing, was inserted through a hole drilled in the top of the cabinet. Suitable connections to the gas sampling tube were made outside the cabinet. Gas components were measured in a Fisher Unitized Gas Analyzer.

It has been common experience that the best bread is produced by taking the sponge 30 to 45 minutes after the breaking time and this procedure was generally followed. The temperature of the sponges at maturity varied between 84° and 87°F (28.9° and 30.6°C). Doughs were mixed for 2 minutes after the cleanup and taken from the mixer at 80°F (26.7°C). Dough time was 10 to 15 minutes. Exceptions to the general procedure will be noted. For proofing, the temperature was 94°F (34.4°C), the relative humidity 90%, and the time 1 hour, 10 minutes. Practically no difference was observed in the rate of proof between cabinet and open doughs. One-pound loaves were baked at

430°F (221.1°C) for 35 minutes in a large rotary oven. Volume was measured and reported as the average of eight loaves. Loaves were carefully scored in accordance with the method of the American Institute of Baking (1939). For brevity, only the total score together with pertinent remarks on the machining characteristics and the bread have been recorded.

Comparison of Cabinet and Open Sponges

It was first necessary to ascertain if the cabinet procedure possessed any advantages as compared to the conventional open sponge method and, if so, to determine the reasons for this superiority.

Table I gives pertinent data on the comparisons of the cabinet and open sponges under varying atmospheric conditions. Even when both temperature and humidity of the dough room are regulated, the cabinet doughs always maintain their superiority. All through these experiments the cabinet doughs took an average of 2% more absorption. The sponges had finer and more uniform pores. At remixing they were drier and broke up more easily, allowing a few minutes shorter mixing time. The outstanding difference between cabinet and open sponges is in the machining. Cabinet doughs are drier, more pliable and extensible, and machine more smoothly with less dusting flour. The bread from the cabinet doughs always scored slightly higher than the bread from the open doughs, mainly due to more uniform, finer grain and thinner cell walls. Also, there is a slightly more tender crumb and better crumb color. No significant difference was noted in loaf volume, symmetry, or break and shred between the two procedures.

Release and Retention of Carbon Dioxide above the Sponge

When temperature and humidity are controlled, in addition to the other variables such as formula, fermentation, and mixing time, there is only one possible difference in the conditions of fermentation between cabinet and open sponges. This difference is merely the atmosphere above the sponge for the 30- to 45-minute period between the breaking time and the time of remixing. As can be seen from the gas analyses given in Table I, little or no carbon dioxide is released until the sponge breaks. In a cabinet the carbon dioxide level reaches approximately 20% within 5 minutes after the break and is maintained or increased from that level during the remaining 25 to 40 minutes, resulting usually in a concentration of 20 to 35% carbon dioxide above the sponge. When a cabinet is not used, the air circulation, the size of the fermentation room, and the number of sponges being fermented all affect the amount of carbon dioxide present above the sponge. In current

TABLE I

COMPARATIVE DATA FOR COMMERCIAL SIZE CABINET AND OPEN SPONGES FERMENTED UNDER VARYING ATMOSPHERIC CONDITIONS [1]

Exp. No.	Type of sponge	Relative humidity %	Temp. at maturity[2]	Gas analysis time after mixing	Machining quality	Volume cc	Total bread score	Remarks
1	Cabinet	75	°F 84°	15 min. 1 hr. 1 hr. 30 min. 2 hr. 2 hr. 30 min. 3 hr. %CO_2 0.0 0.0 0.1 0.7 18.4 26.8 %O_2 20.8 20.8 20.8 20.7 17.1 15.0 %N_2 79.2 79.2 79.1 78.6 64.5 58.2	Smooth, slightly wet.	2790	95.0	
2	Open	55	84°	15 min. 1 hr. 1 hr. 30 min. 2 hr. 2 hr. 30 min. 3 hr. 3 hr. 30 min. 4 hr. %CO_2 0.0 0.0 0.0 0.2 0.4 10.0 13.0 16.2 %O_2 20.7 20.7 20.6 20.8 20.3 18.7 18.2 17.4 %N_2 79.3 79.3 79.4 79.0 79.3 71.3 68.8 66.4	Smooth, slightly wet and soft.	2810	93.0	Open grain.
3	Cabinet	76	84°	2 hr. 15 min. 2 hr. 40 min. 3 hr. 15 min. %CO_2 0.2 6.6 31.8 %O_2 20.6 19.0 13.6 %N_2 79.2 74.4 44.6	Excellent.	2855	96.5	
4	Open	90	84°	2 hr. 15 min. 2 hr. 35 min. 3 hr. 15 min. %CO_2 0.0 0.6 10.6 %O_2 20.5 20.0 18.4 %N_2 79.5 79.4 71.0	Not as extensible as Exp. 3.	2835	94.0	Sponge was wetter than Experiment 3. Pores not as fine or smooth.
5	Open	100	86°	2 hr. 45 min. 3 hr. 3 hr. 15 min. %CO_2 10.0 12.6 11.0 %O_2 11.6 — — %N_2 78.4 — —	Closer to cabinet but shorter at rounder and molder. Trifle wet at molder.	2540	94.5	Slightly open grain, wet texture.
6	Cabinet	80	85°	2 hr. 45 min. 3 hr. 3 hr. 15 min. %CO_2 27.0 27.6 27.6	Excellent—drier than Exp. 5.	2600	97.0	More tender crumb than Experiment 5.
7	Cabinet with oxygen.	86	86°	1 hr. 2 hr. 2 hr. 25 min. 2 hr. 40 min. %CO_2 0.0 18.0 32.5 34.2 %O_2 52.5 40.8 32.3 34.4 %N_2 47.5 41.2 35.2 31.4	Wet out of rounder and at end of intermediate proof. Short at molder.	2750	89.0	Sponge was sticky, coarse, and "dead." Absorption at dough stage was 3% below standard. Bread had open, coarse grain, crumbly texture, and poor flavor and taste.

TABLE I—*Continued*

TABLE I—*Continued*

Exp. No.	Type of sponge	Relative humidity %	Temp. at maturity[1]	Gas analysis time after mixing	Machining quality	Volume cc	Total bread score	Remarks
8	Cabinet with nitrogen.	80	°F 84°	1 hr. 2 hr. 2 hr. 45 min. 3 hr. 3 hr. 15 min. %CO_2 0.2 0.6 13.5 16.4 19.2 %O_2 20.0 16.8 15.9 14.6 14.0 %N_2 79.8 82.6 70.6 69.0 66.8	Soft and sticky at divider and molder.	2680	91.0	7% lower absorption. Longer mixing time at dough stage until smooth. Slightly open grain.
9	Open in CO_2 filled room.	78	84°	1 hr. 2 hr. 2 hr. 30 min. 3 hr. 15 min. %CO_2 8.6 8.3 9.2 8.4 %O_2 18.8 18.7 18.8 18.8 %N_2 72.6 73.0 71.0 72.8	Smoother than the regular open sponge, still not as dry or mellow as cab. doughs.	2630	95.5	Better grain and more tender crumb than open sponges without CO_2 addition.
10	Cabinet in CO_2 filled room as in Exp. 9.	72	84°	Atmosphere in room as in Exp. 9. Inside cabinet at 3 hr. 15 min. %CO_2 32.4 %O_2 14.0 %N_2 53.6	Excellent.	2740	96.5	Very tender crumb.
11	Open in CO_2 filled room.	72	84°	1 hr. 2 hr. 2 hr. 45 min. 3 hr. 15 min. %CO_2 6.8 11.0 17.4 19.4 %O_2 19.0 18.2 17.0 16.6 %N_2 74.2 70.8 65.6 64.0	Almost equal to cabinet doughs. Better machining than Exp. 9.	2640	95.0	
12	Cabinet in CO_2 filled room as in Exp. 11.	78	86°	Atmosphere in room as in Exp. 11. Inside cabinet at 3 hr. 15 min. %CO_2 43.8 %O_2 11.2 %N_2 45.0	Dry, mellow, elastic, better handling than Exp. 10.	2790	96.5	
13	Open in CO_2 filled room.	89	86°	1 hr. 2 hr. 45 min. 3 hr. 3 hr. 15 min. %CO_2 31.4 21.5 20.6 20.6	Excellent—equal to cabinet doughs.	2560	96.5	Slightly open grain.
14	Open in CO_2 filled room.	62	86°	1 hr. 2 hr. 2 hr. 45 min. 3 hr. 3 hr. 15 min. %CO_2 6.2 7.6 32.0 45.4 27.6[3] %O_2 19.3 18.9 13.8 10.8 14.0 %N_2 74.5 73.5 54.2 43.8 58.4	Excellent—equal to cabinet doughs.	2600	96.5	

[1] Room temperature 80° F (26.7° C).
All sponges were mixed 5 minutes at 65 rpm.
All sponges were 78° F (25.6° C) when taken from the mixer, except in Exp. 7 where temperature was 77° F (25.0° C).
All doughs were mixed 2 minutes after cleanup.
[2] 84° F = 28.9° C; 85° F = 29.4° C; 86° F = 30.0° C.
[3] Tank went empty at 3 hours 5 minutes.

practice this amount of carbon dioxide is much less than that held above the cabinet sponges; perhaps 5 to 10% carbon dioxide would be a fair estimate. If the fermentation room has a low ceiling, if there is only slight air circulation, or if there is little space relative to the weight of dough being fermented, there will be relatively more carbon dioxide in the atmosphere than would otherwise be the case. As an illustration, on a laboratory scale, doughs made from 10 pounds of flour (6 pounds of flour in the sponge) were fermented by the cabinet and open procedures (with 2% yeast and the same formula as given for the large doughs) in a Bailey-Walker cabinet, maintained at 80°F (26.7°C) and 75% relative humidity. In the Bailey-Walker cabinets there is quite an extensive air circulation. The louvers were open just sufficiently to allow the temperature and humidity to be held constant. Gas analyses gave the results shown in Table II.

TABLE II

COMPARATIVE COMPOSITION OF ATMOSPHERE OVER LABORATORY SCALE CABINET AND OPEN SPONGES FERMENTED IN A BAILEY-WALKER CABINET (MAINTAINED AT 80°F (26.7°C) AND 75% RELATIVE HUMIDITY)

	Cabinet	Open	Cabinet	Open
Breaking time of sponge	2 hr. 30 min.	3 hr. 40 min.	2 hr. 45 min.	3 hr. 45 min.
Gas analyses at:				
2 hr. 25 min.	$\%CO_2$ 1.0 $\%O_2$ 20.6 $\%N_2$ 78.4	$\%CO_2$ 0.0 $\%O_2$ 20.8 $\%N_2$ 79.2	—	—
2 hr. 30 min.	—	—	$\%CO_2$ 0.6 $\%O_2$ 20.0 $\%N_2$ 79.4	—
2 hr. 35 min.	$\%CO_2$ 12.5 $\%O_2$ 17.7 $\%N_2$ 69.8	—	—	—
2 hr. 45 min.	—	—	$\%CO_2$ 2.3 $\%O_2$ 20.0 $\%N_2$ 77.7	—
3 hr.	—	—	$\%CO_2$ 22.4 $\%O_2$ 15.9 $\%N_2$ 61.7	—
3 hr. 15 min.	—	—	$\%CO_2$ 23.4 $\%O_2$ 15.8 $\%N_2$ 60.8	—
3 hr. 45 min.	$\%CO_2$ 19.5 $\%O_2$ 14.9 $\%N_2$ 65.6	$\%CO_2$ 0.2 $\%O_2$ 20.7 $\%N_2$ 79.1	—	$\%CO_2$ 8.0 $\%O_2$ 19.0 $\%N_2$ 73.0
4 hr. 30 min.	$\%CO_2$ 25.4 $\%O_2$ 15.4 $\%N_2$ 59.2	$\%CO_2$ 1.5 $\%O_2$ 20.3 $\%N_2$ 78.2	—	$\%CO_2$ 1.0 $\%O_2$ 20.4 $\%N_2$ 78.6

As usual, gas samples were taken approximately 1 inch above the sponge. The highest carbon dioxide obtained above any of the open sponges was 8%. A few minutes later most of the carbon dioxide was swept out. On the other hand, the cabinet doughs naturally confined the liberated carbon dioxide above the sponge. In a series of four such comparisons, the open sponge did not break until about 3 hours, 40 minutes, even though the humidity was 75%. There was sufficient circulation of air to cause some thickening of the surface layer of the sponge, although there was no noticeable crusting. The best bread was produced by allowing a sponge time of 4½ hours for the open sponges. The cabinet sponges broke between 2 hours, 30 minutes to 2 hours, 45 minutes and the best bread was produced with a sponge time of 3 hours, 15 minutes. The cabinet dough had superior handling properties whether the sponges were fermented for 3 hours or 4 hours. Conditions such as the size of the doughs and an air circulation sufficient to cause crusting are obviously different from those maintained in good commercial practice. These data are cited merely to show the effect of too much air circulation on the carbon dioxide content of the air above the sponges. In the large commercial doughs, on the other hand, breaking time is the same for both cabinet and open fermentations if the sponges are fermented at a high enough humidity.

In the fermentation rooms of well-equipped bakeries, there is not usually the extensive forced air circulation that exists in our standard laboratory fermentation units. On laboratory size doughs, results more in accord with cabinet fermentation are, of course, achieved when sponges are covered.

Effect of Relative Humidity on Breaking Time of the Sponge

When open sponges are fermented in a room where the humidity is under 70%, the sponge takes a longer time to break and the breaking time is in direct relation to the relative humidity. A low relative humidity in the dough room necessitates a longer sponge time if judged by the time of break. From 70 to 100% relative humidity the breaking time is about the same. Apparently greater humidity makes the surface of the sponge weaker and more pliable; hence it breaks more quickly and evenly with a given amount of pressure to permit the release of the gas generated during fermentation. Experiments 1 through 5 in Table I illustrate this. The open sponge in Experiment 2 at 55% relative humidity broke unevenly in 3 hours, 5 minutes; whereas the cabinet sponges (75 to 82% humidity) and the open sponges, fermented at 75 to 100% humidity, broke in practically the same time, 2 hours, 30 minutes, to 2 hours, 40 minutes. It is recognized that the optimum sponge time need not be estimated by such

an arbitrary procedure as the one we have followed, for, provided a certain minimum time is given, bread quality is not greatly influenced by variations in sponge time.

One great advantage of cabinets lies in their ability to control humidity in small shops having no well-regulated fermentation room. A dough put in a cabinet directly from the mixer gives off enough moisture to keep the humidity at a fairly constant and optimum level. Also, as Schoonover, Freilich, and Redfern (1946) have shown, cabinets effect some degree of temperature control in rooms where the temperature is below or above the temperature of the sponge. Since many small and medium size shops have inadequate control of temperature and humidity, the use of cabinets to effect some means of control of these important factors is of great value.

Effect of Carbon Dioxide, Oxygen, and Nitrogen Atmospheres over the Sponge

Comparisons between cabinet and open sponges indicated that there could be no factor responsible for the differences observed in dough properties except the concentration of gases above the sponges between the breaking time and the time of remixing—a period of 30 to 45 minutes. At first thought it seemed hardly likely that a gas could diffuse to any extent into the sponge, due to the positive pressure of the carbon dioxide being liberated as a result of fermentation. Rather, it was believed that if the carbon dioxide tension on the outside of the sponge was increased, it might prevent some diffusion of the carbon dioxide from the dough, thus building up, after the initial liberation, a greater concentration of the gas inside the dough. Then it would be possible that the higher amount of carbon dioxide might exert an influence on certain enzyme reactions occurring during the later stage of the sponge fermentation.

In order to secure more information on the permeability of the dough to gases, fermentation was conducted in atmospheres higher than normal in both oxygen and nitrogen. Cabinets were placed over the sponges and the gases introduced into the cabinet immediately after the sponges were taken from the mixer. Results of the gas analyses and the baking data are recorded in Table I, Experiments 7 and 8.

When oxygen was used in the cabinet, the sponge was coarse, sticky, and "dead." At the dough stage more flour was required for the same amount of water, resulting in 4% lower total absorption. The dough was wet out of the rounder and at the end of the intermediate proof. The dough coming from the molder was short. The bread exhibited an excellent oven spring, but had an open grain and a crumbly

texture. The aroma, flavor, and taste were flat. Gas analyses at the end of the sponge period demonstrated as high a carbon dioxide content as was found with the regular cabinet sponge. However, the oxygen content of the gas above the sponge was about double and the nitrogen approximately half that shown by the cabinet sponge.

When nitrogen was used in the cabinet, absorption at the dough stage was even less than with the cabinet sponge fermented in the atmosphere containing excess oxygen and 7% lower than the regular cabinet sponge. It was found necessary to mix 6 minutes longer than normally to secure a smooth dough. The dough was soft and sticky through the machines. Although oven spring was good, the loaf representing the sponge fermented in excess nitrogen gave an open grain, a gummy texture, and very poor aroma, taste, and flavor. The atmosphere above the sponge during the last 30 minutes of the sponge period showed a slightly lower carbon dioxide and oxygen content; the nitrogen percentage was higher throughout the sponge period. Some objection might be raised because oxygen and nitrogen were introduced at the beginning of the sponge period. However, similar experiments in which carbon dioxide was introduced at the start of the sponge period gave results equal to, or better than, the usual cabinet doughs.

These experiments demonstrate that the sponge must be permeable to the gases above it and that high concentrations of nitrogen and oxygen cause damage to the physical characteristics of the dough and the resultant bread, as Johnson (1945) has previously shown on smaller doughs.

It is interesting to note, in this connection, the results reported by Baker and Mize (1937) on the effect of mixing doughs in vacuum and in the presence of various gases. They found that doughs mixed in atmospheres of hydrogen and nitrogen became sticky and soft on long mixing; doughs mixed under oxygen developed, in addition, pronounced shortness. However, normal bread was produced from the doughs subjected to mixing in vacuum or in the presence of hydrogen or nitrogen. Doughs mixed in the presence of oxygen gave improvement followed by pronounced deterioration.

Experiments were then conducted to see if open sponges fermented in a room filled with higher than a normal concentration of carbon dioxide gave results similar to the cabinet procedure (Table I, Experiments 9 to 14 inclusive). Carbon dioxide was introduced by attaching a tank to the humidifying system of the fermentation room which was 7 feet high; 5 feet, 9 inches long; and 6 feet, 2 inches wide. Gas samples were taken at the level of the trough by means of tubing inserted through the roof of the room. In Experiment 9 the carbon dioxide in the room varied from 8.3 to 9.2% throughout the sponge period.

A cabinet sponge placed in the room at the same time showed 32.4% carbon dioxide when the sponge was taken. This open sponge was slightly smoother than the regular open sponges but was not as dry and mellow in handling as the regular cabinet dough. The cabinet dough fermented in the room at the same time had excellent machining quality. Experiments 11 and 12 were conducted at a higher level of carbon dioxide. During the last 30 minutes of the sponge period, between 17.4 and 19.4% carbon dioxide was maintained. The open sponge was almost equal to the cabinet sponges in machining and bread quality. The cabinet sponge fermented at the same time in this carbon dioxide-filled room attained a level of 43.8% carbon dioxide. This was the highest figure found in a cabinet sponge and the dough had the best machining characteristics of all the cabinet doughs. In Experiment 13 the carbon dioxide concentration was again increased. At the end of 1 hour it was 31% and thereafter, during the critical period, about 21%. The dough was equal to the cabinet sponges. In Experiment 14 the carbon dioxide was maintained at a still higher level—between 28 and 45% from 2 hours, 45 minutes to 3 hours, 15 minutes. This dough was also equal to that obtained from the cabinet procedure in handling characteristics and in bread quality; thus it would appear that approximately 20% carbon dioxide, with proportionate lowering of oxygen and nitrogen, during the last 30 to 45 minutes of the sponge time is the critical level for the production of excellent machining and superior bread.

Under the conditions of these experiments it was not feasible to ferment a dough in a room where the carbon dioxide was maintained at a high enough concentration for only the last 30 to 45 minutes of the sponge period. In order to build up a sufficient concentration of carbon dioxide in the dough room, it was found necessary to introduce the carbon dioxide from the beginning of the sponge time. However, a rather interesting experiment was tried in which two sponges were fermented under exactly the same conditions of mixing, fermentation time, and temperature, one open and the other with a cabinet on top of the trough. At 2 hours, 25 minutes (just before the break) the cabinet was taken off the cabinet sponge and put on the open sponge. A sponge time of 3 hours, 15 minutes was given both doughs. It was found that the sponge that was covered with the cabinet during the last 50 minutes had superior machining characteristics to the sponge that was open during the last 50 minutes but covered prior to that time.

The machining characteristics comprise the outstanding difference between doughs made from cabinet and open sponges, and it is unfortunate that they cannot be properly evaluated by any single

chemical or physical means, but must be judged by the feeling of the dough at the divider, rounder, and molder. Since the farinograph and the extensograph are the only means available to record certain differences in the physical properties of doughs, the following experiments were conducted.

Farinograph and Extensograph Measurements

What change does the higher concentration of carbon dioxide cause during the short interval it is present above the fermenting sponge? As the first step in elucidating this problem, extensograms and farinograms were made on the sponges, and on the doughs after remixing. A different flour from that previously employed was used in this series of experiments, but it showed the same analyses as the flour previously reported. The formula was the same as that described for the experiments listed in Table I and both open and cabinet sponges and doughs were handled at the same absorption. The open sponges were fermented in the small dough room maintained at a relative humidity of 82 to 85% and a temperature of 80°F (26.7°C). Sponges were taken from the mixer at 78°F (25.6°C) after a 5-minute mix and the doughs were mixed for 2 minutes after the cleanup. The cabinet doughs needed less mixing. This procedure was justified because in usual commercial practice doughs are mixed for a definite time after the cleanup. The fermentation times chosen for the open sponges were $3\frac{1}{4}$, 4, and $4\frac{3}{4}$ hours, and the best bread was produced with the 4-hour sponge time. Two cabinet doughs were compared with the open sponges, using sponge times of 2 hours, 55 minutes and 3 hours, 15 minutes. Previous experiments with other flours had shown that 3 hours, 15 minutes was ample time for a cabinet sponge. The relative humidity inside the cabinet was 85%. The baking data are presented in Table III.

Duplicate farinograph and extensograph curves were conducted on all samples and good checks were obtained. A weight of 480 g was used for the farinograph curves and 150 g for the extensograms. Farinograms were made on pieces of the sponge taken just before remixing and again on the dough immediately after remixing. The samples for the extensograms were rounded and molded in the usual fashion. Measurements were made immediately on the rounded and molded sponge, but on the remixed dough a rest period of 38 minutes was given. This interval was chosen because it corresponds closely to the time actually taken between the remixing and molding of the large doughs. It is evident that the rounding and molding manipulations in the extensograph change the state of the dough and measure certain physical characteristics of the dough relatively at rest as com-

pared with the farinograph where the gluten strands are subjected to continuous mixing. The length of the extensogram indicates the extensibility, and the height of the curve indicates the resistance of the dough to extension, or the energy necessary to stretch the dough. For best breadmaking results, a proper balance should exist between the length and height of the curve. The interpretation of farinograms is too well known to discuss here.

Figure 1 illustrates the results obtained with the farinograph and the extensograph. The farinograph curves A, B, and C on the open sponges showed the highest consistency and the greatest strength on the $3\frac{1}{4}$-hour sponge. The 4- and $4\frac{3}{4}$-hour sponges became progressively weaker, as shown by both the amplitude and the breaking point of the curves. The curves on the 2 hour, 55 minute and $3\frac{1}{4}$-hour-cabinet sponges (D and E) and the $3\frac{1}{4}$-hour open sponge (A) were not significantly different, D showing somewhat more strength. Farinograph curves I and J on the cabinet doughs, however, show a definitely higher consistency than the curves (F, G, and H) on the doughs made from all the open sponges. This was not due to the few minutes shorter mixing time of the cabinet sponges at the dough stage, since other experiments have shown that a high consistency on the farinograph curves was obtained when the mixing times on both open and cabinet doughs were identical. Numerous comparisons of laboratory or commercial size cabinet and open sponges under controlled conditions of temperature and humidity have always shown that a drier dough is given by the cabinet method.

At the end of the fermentation period, all sponges are "short"; this is illustrated by the extensograph curves K through O. The curves on the open sponges all indicate about the same extensibility. The height of the curves decreased progressively from 360 to 310 to 280 units as the fermentation time increased. Curve O for the cabinet sponge with a fermentation time of 3 hours, 15 minutes exhibited the most extensibility and the greatest resistance to extension (420 units) of all the curves. The cabinet sponge fermented for 2 hours, 55 minutes, however, gave a curve (N) very similar to the 4-hour open sponge, the only difference being that the cabinet curve was 20 units higher. The extensograms on the doughs are much easier to interpret since conditions more closely parallel the state and time of the dough at actual machining. The extensograph curves S and T of both cabinet doughs gave a greater area than the curves P through R obtained from any of the open sponges. Results in machining verify these measurements since the cabinet doughs machined more smoothly and were drier and more mellow than any of the open sponges.

These results, in conjunction with the baking data given in Table

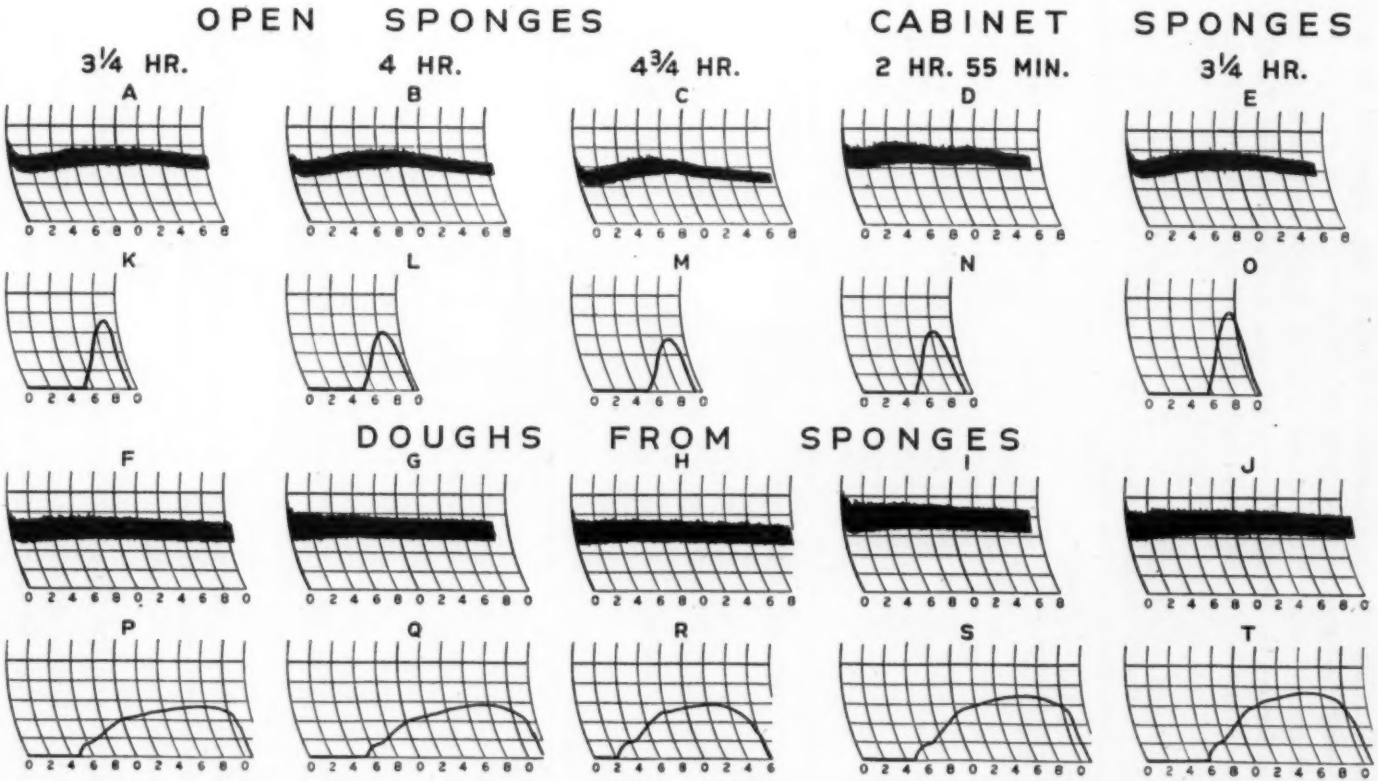

Fig. 1. Farinograph and extensograph curves of sponges and doughs.

III, substantiate the conclusion that shorter sponge times are possible when using the cabinet procedure.

Farinograph and extensograph curves were also made on the sponges fermented in oxygen and nitrogen and reported in Table I, but space does not permit their inclusion. It was interesting to note

TABLE III

COMPARATIVE BAKING DATA FOR CABINET AND OPEN SPONGES EMPLOYED IN EXTENSOGRAPH AND FARINOGRAPH STUDIES[1]

Exp. No.	Type of sponge	Sponge time	Temp. of sponge at maturity[2]	Machining quality	Volume cc	Total bread score	Remarks
			°F				
15	Open	3 hr. 15 min.	85	Dough mixed smooth but was a trifle soft. No sign of shortness in mixer but was short at divider. Recovered in overhead proofer. Smooth and pliable at molder. Normal proof.	2530	90.5	Crumb was short and slightly gummy.
16	Open	4 hr.	87	Dough mixed smooth but was a trifle soft. No shortness in mixer or at divider. Molded somewhat tough. Not as smooth as usual doughs. Normal proof.	2630	92.5	Slightly open grain.
17	Open	4 hr. 45 min.	87	Sponge was a trifle soft. Dough mixed smooth, soft, and a trifle wet. Some shortness at divider. Molded a little tough. More gas than usual in dough at molder.	2790	90.5	Slightly open grain with a few large holes. Wet and crumbly texture.
18	Cabinet	2 hr. 55 min.	84	Sponge shorter and drier than open sponges. Dough mixed smooth in 4 min. less than open sponges. Machined easily. Dry at divider. Molded very smooth.	2595	95.5	Good grain, thin cell walls, silky texture.
19	Cabinet	3 hr. 15 min.	85	Sponge shorter and drier than open sponges. Mixed smooth in 5 min. less mixing than open sponges. Machined easily. Dry at divider and molder.	2630	95.5	Slightly open grain, thin cell walls, silky texture.

[1] Room temperature, 80°F (26.7°C); relative humidity of dough room, 82 to 85%; relative humidity inside cabinet, 85%. Breaking time of all sponges was between 2 hours, 30 minutes and 2 hours, 40 minutes.

[2] 84°F = 28.9°C; 85°F = 29.4°C; 87°F = 30.6°C.

that both the farinograms and extensograms on the doughs made from sponges fermented under excess oxygen and nitrogen indicated poor handling properties.

Hydrogen Ion Concentration of Open and Cabinet Sponges

Although it would not be expected that there would be a difference in the hydrogen ion concentration of the open and cabinet sponges, since there appeared to be no difference between them until the break-

ing point, this factor was checked on the two sponges fermented under identical conditions of formula, temperature, and time. The only difference, a negligible one, was that the relative humidity inside the cabinet was 83%, while in the dough room, where the open sponge was fermented, it was 76%. Glass and calomel electrodes were inserted in the sponge and the changes in hydrogen ion concentration with time were measured with a Cambridge potentiometer. Results were as follows:

Time after mixing sponge	pH Open	pH Cabinet
Initial	5.7	5.7
15 min.	—	5.5
25 min.	5.6	—
45 min.	5.3	5.3
1 hr. 15 min.	5.2	5.3
1 hr. 50 min.	5.1	5.2
2 hr. 5 min.	—	5.1
2 hr. 20 min.	5.1	5.1
3 hr.	5.1	5.1
3 hr. 15 min.	5.1	5.1

It is evident that no difference in hydrogen ion concentration or its rate of change is responsible for the superior machining of the cabinet doughs.

Carbon Dioxide Production under Different Gas Atmospheres

So far there is ample proof that the only condition which could cause the differences observed in the machining of the dough and in the quality of the bread between cabinet and open sponges is the higher concentration of carbon dioxide present above the cabinet sponge during the interval between the breaking of the sponge and its remixing. What is the mechanism of the action of this increased level of carbon dioxide on the sponge? There is a possibility that during this last stage of the sponge fermentation the higher level of carbon dioxide confined over the cabinet dough causes a more rapid generation of gas. This higher concentration of carbon dioxide may then act favorably on certain enzyme systems or on the gluten itself.

To obtain more accurate information on the effect of various gases on the rate of carbon dioxide production by the action of yeast on flour, this phase of the subject was investigated using the Warburg apparatus. Details concerning the principle and the operational techniques have been described by Dixon (1943) and Umbreit, Burris, and Stauffer (1945). Since a free exchange of gases is essential in employing any type of respirometer, it is necessary to employ more dilute suspensions than exist in dough. The suspensions were made up as follows: 50 ml of water or buffer were added to 10 g of the short patent flour employed for the baking procedures, and the suspension

was thoroughly mixed for about 2 minutes with a mechanical stirrer. A quantity of 2 ml was used. A volume of 1 ml of the yeast suspension (0.4 g fresh compressed yeast made up to 50 ml with water or buffer) was employed, thus giving a concentration of about 2% yeast based on the flour weight. In all experiments blanks were conducted on the flour and yeast suspensions separately. Each of the blanks was made up to 3 ml with water or buffer and the sum of the two blanks was subtracted from the readings of the flour-yeast mixture. The yeast solution was tipped into the flour suspension from the side arm after 15 minutes' equilibration. A temperature of 37°C was used throughout. In all the experiments where the fermentation was studied with gases other than air, the three flasks with flour alone, yeast alone, and flour and yeast were gassed for 10 minutes before placing them in the bath. The gas was bubbled through water before its introduction in the flasks. A similar series with air was always conducted at the same time. The manometers were then set at 150 after equilibration and readings of the carbon dioxide liberated taken at suitable intervals. Figure 2 illustrates average results obtained using water, rather than a buffer, with flour-yeast mixtures fermented in air, carbon dioxide, nitrogen, and oxygen. Water was used because it simulates more closely the conditions present in dough.

It would be expected that the hydrogen ion concentration of the flour-yeast suspensions gassed with carbon dioxide would be lower than the samples gassed with oxygen or nitrogen. The flour suspension in the main compartment and the yeast suspension in the side arm were gassed for 10 minutes in the usual fashion with carbon dioxide, oxygen, and nitrogen. An air control was used for comparison. The contents of the side arm were added to the flour suspension and the hydrogen ion concentration measured immediately with microelectrodes. Results were as follows:

	pH
Flour extract alone	5.82
Flour-yeast with air	5.79
Flour-yeast with nitrogen	5.66
Flour-yeast with oxygen	5.64
Flour-yeast with carbon dioxide	5.06

The higher hydrogen ion concentration of the flour-yeast mixtures fermented in air or oxygen as compared to carbon dioxide explains the delayed start of the curves in air and in oxygen. Until the water is saturated with the carbon dioxide produced by fermentation, there is no liberation of gas. The solubility of carbon dioxide at 37°C is 0.57 ml carbon dioxide per ml water, which is significantly higher than the other gases employed. Flour-yeast suspensions in water fermented under carbon dioxide and nitrogen gave increased amounts of carbon

dioxide as compared to air and oxygen, fermentation in nitrogen being much faster owing, perhaps, to the more rapid diffusion of this gas.

Because of the differences in the initial hydrogen ion concentration of the flour-yeast-water mixtures, it was of interest to repeat the experiments substituting a sodium acetate-acetic acid buffer of hydrogen ion concentration 5.0 for the water. Both the flour and yeast sus-

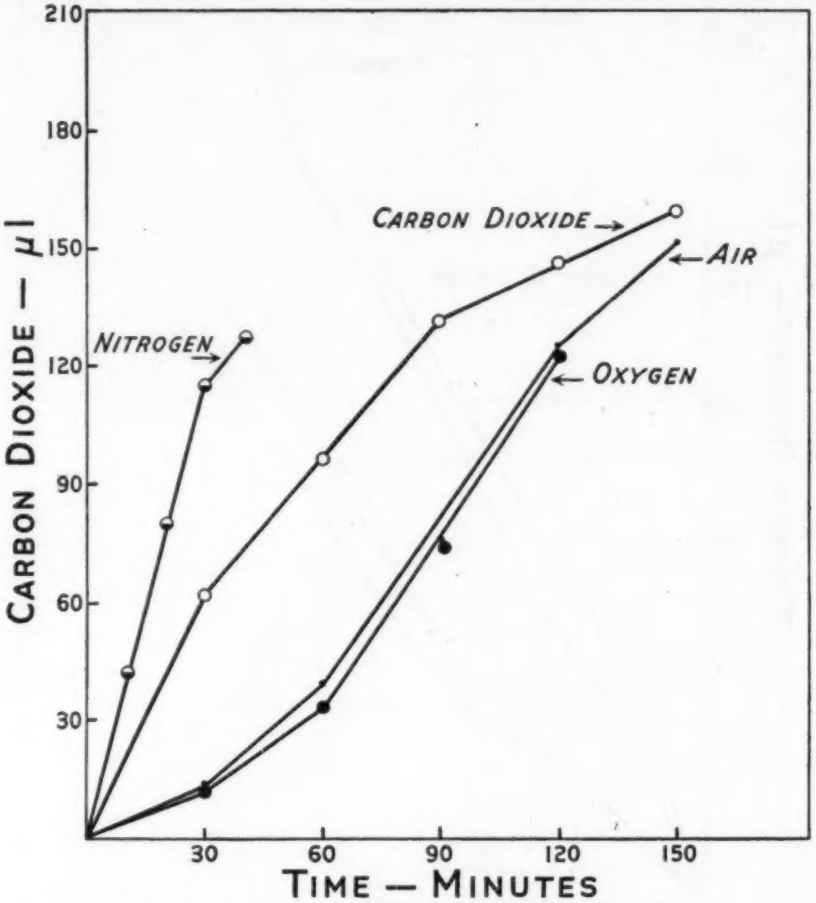

Fig. 2. Carbon dioxide production of flour-yeast water suspensions fermented in air, oxygen, nitrogen, and carbon dioxide.

pensions were made up with this buffer, which had approximately the same hydrogen ion concentration as existed at the start of the carbon dioxide experiment and was also the hydrogen ion concentration found at the end of all the Warburg experiments. Figure 3 illustrates the average results. Because of the lessened solubility of carbon dioxide at the lower hydrogen ion concentration, there is not the usual induction period found when water is used as the medium in the air and oxygen

curves. The carbon dioxide and nitrogen experiments gave about the same gas production in the buffered suspensions and, as might be expected, both were higher than when the fermentation was conducted in air or oxygen.

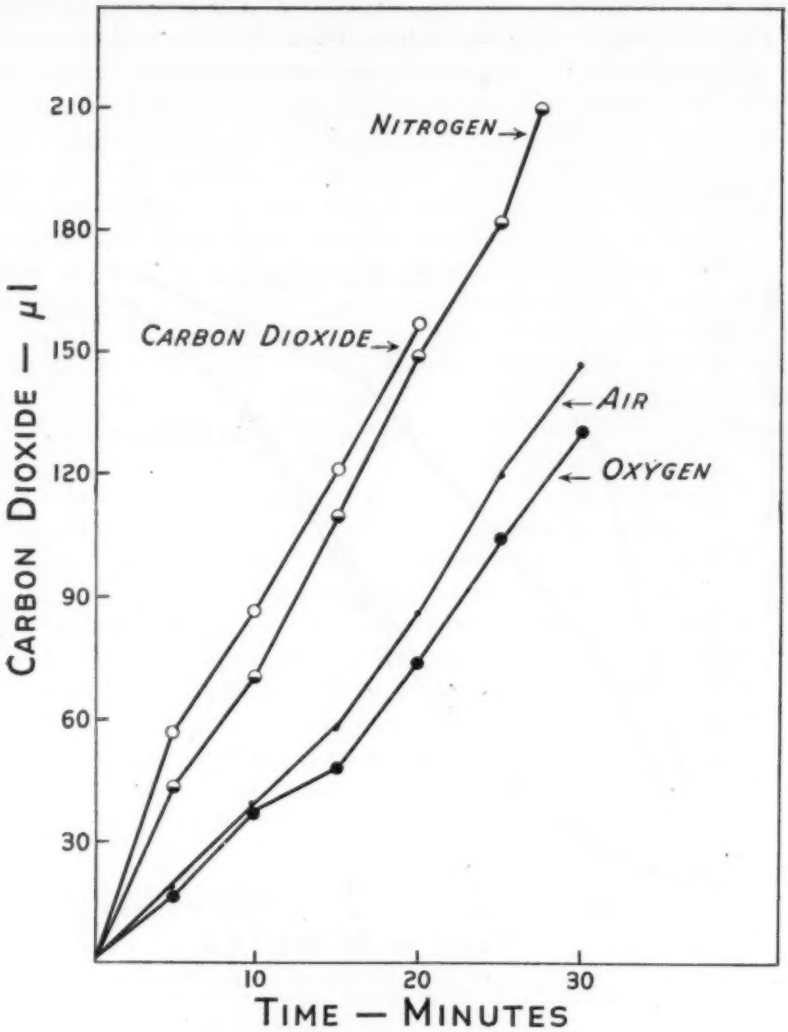

Fig. 3. Carbon dioxide production of flour-yeast-buffer (pH 5) suspensions fermented in air, oxygen, nitrogen, and carbon dioxide.

These experiments show that gas production of flour and yeast suspensions is more rapid in an anaerobic atmosphere.

Maltose and Sucrose in the Fermenting Sponges

A sponge was mixed using the ingredients given in the original formula, except that no yeast food was included. After a 5-minute

mix the sponge was divided; one-half was fermented in air, the other in a cabinet into which carbon dioxide was conducted. Maltose was determined on 10 g of the sponge by the Blish-Sandstedt procedure, as modified by Sandstedt (1937), at various stages of fermentation. Figure 4 shows that the sponge fermented under carbon dioxide had

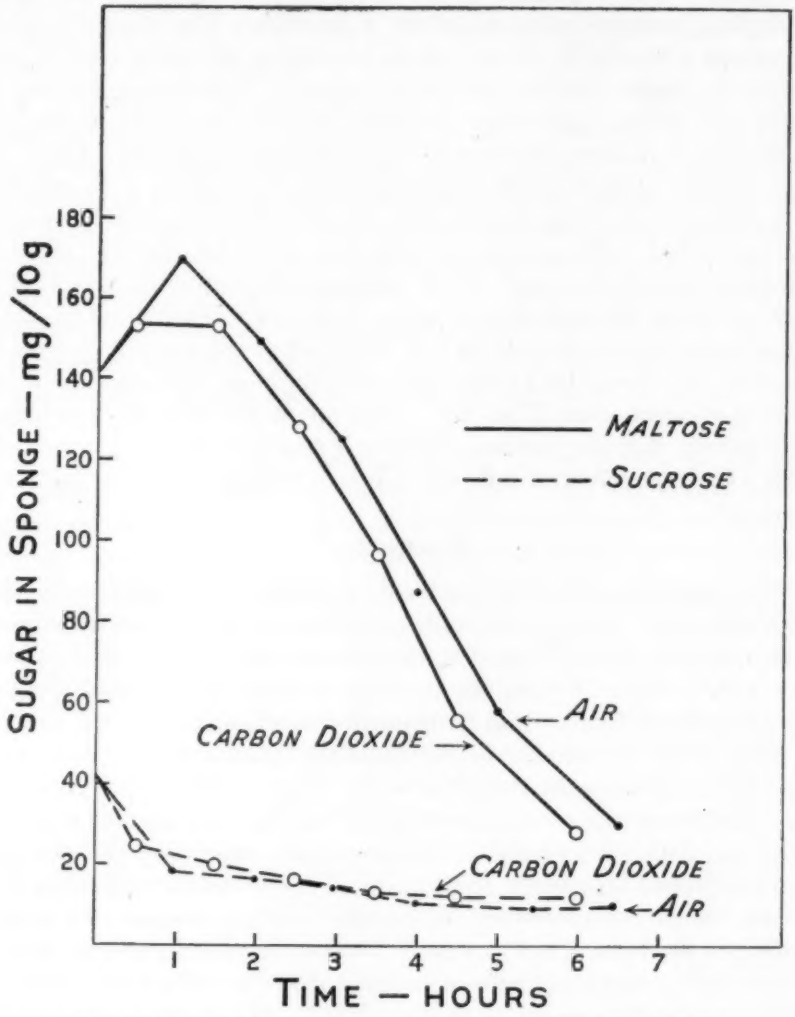

Fig. 4. Maltose and sucrose in sponges fermented in air and carbon dioxide.

less reducing sugar at all stages than the sponge fermented in air. The initial increase in maltose around the first hour is always greater in air than in the carbon dioxide-fermented doughs.

Sucrose was likewise determined and its decrease with time is about the same for both doughs.

Retention of Carbon Dioxide in the Cabinet and Open Sponges

As a preliminary check on the amount of carbon dioxide actually contained in the sponges, the following experiment was conducted. Two sponges (39 pounds of flour, 24 pounds of water, 1 pound, 5 ounces of yeast, 1 pound, 15 ounces of shortening, and 0.004% of potassium bromate) were mixed for 5 minutes. The temperature of the sponges was 78°F (25.6°C) from the mixer and 84°F (28.9°C) at maturity. Both cabinet and open sponges were fermented for 4 hours in a room maintained at 80°F (26.7°C) and 85% humidity. At the end of the fermentation period a piece of dough was taken from each sponge. Duplicate 30-g samples from each sponge were weighed in a 250-ml centrifuge bottle and 60 ml 0.1 *N* barium hydroxide solution added. The samples were shaken for 15 minutes in a shaking machine, centrifuged, and 30-ml aliquots titrated with 0.1 *N* HCl solution using phenophthalein as an indicator. The 30-ml aliquots of the open sponge used 26.45 and 26.53 ml of the barium hydroxide solution (30 ml—ml HCl required), while the same size aliquots of the cabinet sponges used 27.40 and 27.22 ml of the barium hydroxide. This proves that the cabinet sponges actually held more carbon dioxide—the equivalent of 0.82 ml of 0.1 *N* barium hydroxide per 15 g of sponge.

Discussion

It is well known that bakers' yeast does not rigidly conform to one metabolic type. Under anaerobic conditions, fermentation is favored; in the presence of air or oxygen, there is an adaptation to the respiratory type. Many organisms, as well as plant and animal tissues, possess both respiratory and fermentation mechanisms and the faculty of using either the aerobic or the anaerobic system, as the conditions require, is known as the Pasteur effect. Much work has been done on the yeast fermentation of glucose under aerobic and anaerobic conditions, but little or no information is available regarding the effect of such conditions on panary fermentation. Fermentation consists of a number of oxidation-reduction reactions of the trioses which are formed by the cleavage of glucose. These reactions depend on several enzyme systems whose delicate balance may be shifted by means of the gaseous environment, or by the presence of certain compounds or groups capable of interference or protection of some of the energy transfer systems. The -SH groups, for instance, undoubtedly have, as one of their functions, the protection of certain systems against oxygen injury and, hence, act as regulators of the fermentation reactions. Before we can understand the reason for some of the overall effects observed in cabinet fermentation, it will be necessary to

study certain of these specific enzyme systems. It is quite probable that the atmosphere present above the sponge will influence the rate and the course of many of these reactions since, apparently, the sponge is permeable to the gases above it. This permeability will depend on the rate of diffusion of the individual gases and the efficiency of the sponge in acting as a semipermeable membrane. The velocity of diffusion of a gas is in inverse proportion to the square root of its density. If air is taken as 1, the velocity of diffusion of the other gases is nitrogen, 1.014; oxygen, 0.950; and carbon dioxide, 0.812. However, in the case of a moist membrane in which a gas is soluble, its density exerts a minor effect and the rate of diffusion is proportional to the solubility of the gas in the solvent. Thus, carbon dioxide will pass through a moist membrane more rapidly than oxygen or nitrogen, both of which have a higher velocity of diffusion, because carbon dioxide is appreciably soluble in water. The adverse effect produced by fermenting sponges in an atmosphere higher than normal in oxygen and nitrogen indicates that these gases must diffuse into the sponge. The probability is that carbon dioxide diffuses into the sponge to an even greater extent.

It had been shown manometrically that carbon dioxide production by flour-yeast suspensions is higher in the presence of nitrogen and carbon dioxide, a fact which has been proved numerous times on simpler fermentation systems. That oxygen inhibits true fermentation has been known since the time of Pasteur. Likewise, as further proof, it has been indicated that the total sugar content of sponges fermented in carbon dioxide is less than that of sponges fermented in air. Yet fermentation under nitrogen gives most unsatisfactory results and fermentation under carbon dioxide achieves results better than normal. It is evident that carbon dioxide has a specific effect and this effect is much more important than the maintenance of a state of anaerobiosis. Because of the carbon dioxide produced in the fermentation, anaerobiosis is present, to a large and perhaps sufficient degree, in sponges fermenting in air. The increased amount of carbon dioxide presumably present in the sponges from the cabinet procedure during the period after the break and before the remixing of the sponge may exert its effect by combining chemically with the basic amino groups of the protein, or by changing the course of certain enzyme reactions because of its replacement of air in the fermenting dough. The phenomena of carbon dioxide retention by proteins such as serum is well known. As measured by barium hydroxide titrations, a greater carbon dioxide retention was found in the cabinet sponge than in the open sponge. Until more research is done on the specific mechanism of the action of increased concentrations of carbon dioxide on ferment-

ing sponges, it is not possible to explain fully the effects observed. It is planned to continue this work by studying some of the oxidizing enzyme systems present in dough and the influence of carbon dioxide upon them.

Summary

Comparisons of open and cabinet sponges fermented under controlled conditions of temperature and humidity have shown the marked superiority of the cabinet method. This superiority lies mainly in the production of drier, more mellow doughs that machine more smoothly. From 1 to 2% higher absorption may be employed with the cabinet procedure, and optimum mixing time is always a few minutes shorter than with the conventional open sponge fermentation. Likewise, a shorter sponge time may be employed. In the bread, the main advantages of the cabinet method are a softer texture and thinner cell wall. The effect of cabinets in maintaining some degree of temperature regulation and an optimum humidity in shops not too well controlled for humidity and temperature is obvious.

Numerous gas analyses proved that no significant amount of carbon dioxide is liberated until the sponge breaks; therefore the superiority of the cabinet sponges must be due to the greater concentration of carbon dioxide over the sponge during the period between the breaking of the sponge and the time of remixing. The best results were obtained when the critical concentration of carbon dioxide at this period was approximately 20%. Under commercial conditions, seldom, if ever, are open sponges fermented in an atmosphere containing this high a level of carbon dioxide. However, open sponges will give results similar to the cabinet procedure if carbon dioxide is maintained in the dough room at a high enough concentration.

When sponges are fermented in an atmosphere of either oxygen or nitrogen, poor machining and inferior bread result. This would indicate that the sponge is permeable to the gases above it, and because of the greater solubility of carbon dioxide in water, it is probably more permeable to carbon dioxide than oxygen or nitrogen.

Gas production is more rapid when flour-yeast suspensions are fermented in nitrogen or carbon dioxide than in air or oxygen, but this does not explain the better results obtained with cabinets, since gas production is even more rapid in nitrogen than in carbon dioxide. Neither does any difference in hydrogen ion concentration between open and cabinet sponges account for the results obtained. It is suggested that the greater anaerobiosis obtained by higher concentrations of carbon dioxide is of much less importance than a specific effect of carbon dioxide. This effect may be either a combination of carbon

dioxide with basic groups of the protein or the action of carbon dioxide on certain oxidizing enzyme systems present in the fermenting dough, but both possibilities remain to be proved.

Acknowledgments

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FARINOGRAMS AND MIXOGRAMS AS A MEANS OF EVALUATING FLOURS FOR SPECIFIC USES [1]

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Many efforts have been made to devise a mechanical means for measuring and recording the properties of doughs when subjected to continuous mixing. At the present time the two machines most popular in the United States for measuring and recording the behavior of dough during mixing are the Brabender Farinograph and the National Micro Recording Dough Mixer or Mixograph. The development of the farinograph and the mixograph and the principles of their action on dough have been thoroughly discussed in monographs describing various physical dough testing methods which have been prepared by Bailey (1940) and Swanson (1943).

The use of recording dough mixers for testing flour quality and flour classification has been studied by several investigators. Brabender (1932) presented farinograms which show the effect of blending North American hard wheat flours with European soft wheat flours. Brabender (1934) also gives experience covering a period of six years in testing baking value of flours by mechanical means. Munz and Brabender (1940, 1941) demonstrated that farinogram patterns have a definite relationship to type of flour. Geddes, Aitken, and Fisher (1940) made an extensive study of the relation between the farinogram characteristics and baking value of Western Canadian wheat. They concluded that the farinogram characteristics were not as valuable as flour protein as an index of loaf volume but the instrument provided valuable accessory information on such properties as absorption, optimum mixing time, and mixing tolerance. Aitken, Fisher, and Anderson (1944) studied the relationship between farinogram measurements and the commercial grade of the wheats from which the flours were milled; the curve dimensions failed to change consistently with decreasing grade.

The wide variation in dough development curves made on the mixograph and the possibilities of classifying these into types were shown by Swanson (1939). Johnson, Swanson, and Bayfield (1943) studied the correlation of mixogram characteristics with baking results. They concluded that the greatest value of such testing was the obtaining of data that supplements baking data. Harris, Sibbitt,

[1] Contribution No. 125, Department of Milling Industry.

and Banasik (1943), employing baking ingredients and fermentation, obtained mixograms whose properties when expressed by a single figure were highly correlated with loaf volume.

The object of this study was to compare the farinogram and mixogram patterns of flours actually used for specific purposes and to study these patterns in relation to intended types of processing.

Materials and Methods

One hundred and thirty-two flours obtained from flour mills and bakeries located in various parts of the United States and Canada were used. The flours were classified by those supplying the samples. The groups and numbers of samples obtained were as follows: hearth bread (13), bakery (20), topping and doughing (11), family (19), pastry (13), medium quality cake (13), fancy cake (15), specialty flour for pies, cookies, doughnuts, etc. (7), cracker dough (6), cracker sponge (8), and biscuit (7).

The samples, collected over an 8-week period, were stored at 40°F until all were received, at which time they were analyzed for moisture, protein, and ash and the physical dough tests were started. The samples were coded so that during the testing their association with any specific group was unknown to the technician.

Farinograms were made according to the following procedure specified by the Brabender Corporation. The thermostat was maintained at 30°C and the small mixing bowl was used employing 50 g (14% moisture basis) of resifted flour. The absorption for the normal farinogram was determined by a titration curve bringing all doughs to the 500 Brabender unit consistency at point of minimum mobility. Distilled water, only, was employed.

Mixograms were made with the mixograph (National recording dough mixer) operating at 87 rpm with the No. 9 spring setting in an air-conditioned room maintained at 80°F (26.7°C) ± 1°. Thirty-five grams of flour (14% moisture basis) were used with absorptions established from the regression of absorption and protein content previously determined in the laboratory. The farinograph was placed in the same room as the mixograph.

After farinograms and mixograms had been made on all the flour samples, inspection of each group showed considerable variation in curve types, and hence it seemed best to select five farinograms and five mixograms as representatives of each group. The method of selection was by visual inspection of the mixograms and farinograms of each of the groups. First were selected the two curves which represented the extremes in type and the most "typical mean" which was midway between the two extremes. Then two additional curves

were selected each of which represented a curve type intermediate between the extremes and the "typical mean."

Many measurements were taken from the farinograms and mixograms but only a few seemed pertinent to this study. Farinogram and mixogram mixing requirement (minutes of mixing to point of minimum mobility), farinogram valorimeter, and mixogram area and absorption determinations were chosen to aid in studying the flours. Flour protein and ash content were also included. Statistical means, standard deviations, and coefficients of variability were calculated.

The farinogram and mixogram mixing requirements were determined from the number of curved lines traversed by the pen from the start of the curve to point of minimum mobility. The valorimeter value was obtained according to the method furnished by the Brabender Corporation: The farinogram was placed in a special template (Brabender, 1937) designed to give a single figure strength rating of flours by the farinograms. The template consists of two parts. One part is stationary and has a series of exponential lines which are designated with valorimeter values. The other part is movable and cut to fit the arc made by the pen of the farinograph. The movable part is 12 cm in length. To obtain a valorimeter reading, the farinogram is placed under the stationary part so that the beginning of the curve coincides with both the beginning of the stationary template and the 500 unit consistency line. The movable part is then placed over the stationary part so that the fore edge of the movable template coincides with the point of minimum mobility of the farinogram. The valorimeter value is then read from the stationary template at the point where the opposite edge of the movable template bisects the descending slope of the farinogram. The length of time to point of minimum mobility and the slope after this point determine mainly the valorimeter value. The mixogram area was measured with a planimeter for a mixing period of 8 minutes.

Results and Discussion

The various groups of flours were classified into three broad catagories: (1) bread, (2) pastry, (3) cracker and biscuit flours. The experimental results are thus presented by groups in the three categories by the use of three figures and three tables. The data presented in each of the tables represent the corresponding flours depicted in the figures; in addition, means, standard deviations, and coefficients of variability are given for all flours in each group. The statistical means may not be exactly the same as the "typical means," since the statistical means were obtained by averaging the data from all samples in a

group and "typical means" were obtained from curves chosen by observation of general characteristics.

Bread Flours. The farinograms and mixograms of the four bread flours are presented in Figure 1. Each set of curves within any one group are arranged from left to right according to increasing strength [2] as indicated by increasing length of mixing requirements, decreasing sensitivity to overmixing, increasing width of band, and general dough

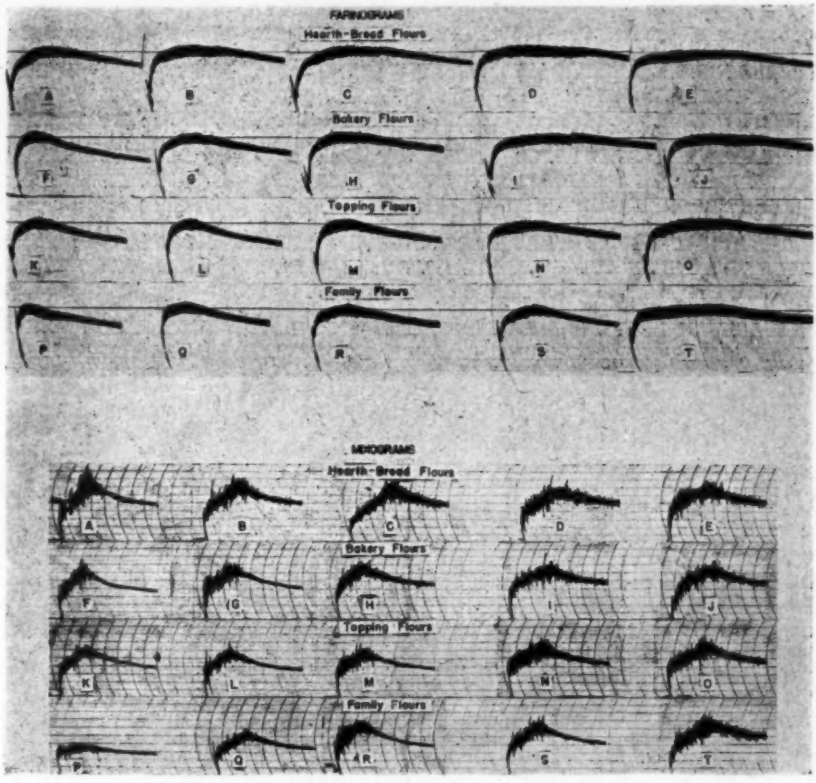

Fig. 1. Farinograms and mixograms for bread flours.

development characteristics. The letter under the peak of each curve designates a definite flour. Data with the identifying letters for the corresponding samples are presented in Table I. Neither the farinograms nor mixograms exhibit sharp lines of demarcation between groups but merge gradually from one to another type (Figure 1). The curves for the strongest flours in each respective group tend to be more alike than those for the weakest flours. The curves of the hearth and bakery flours are more uniform than the topping and family flours.

[2] This defines "strength" as used in this paper.

TABLE I

SUMMARY OF DATA ON BREAD TYPE FLOURS

Fig. 1 Letter	Protein	Ash	Mixogram mixing time	Farinogram mixing time	Mixogram absorption	Farinograph absorption	Mixogram area	Farinograph valorimeter reading
	%	%	*min.*	*min.*	%	%	*cm* [2]	*unit*
Hearth flours—13 samples								
A	13.2	.40	2.5	7.5	64.0	64.5	93.6	66
B	13.5	.47	3.0	6.0	64.0	63.5	94.3	70
C	15.2	.48	3.5	8.5	66.0	68.0	85.2	77
D	13.3	.76	3.2	9.0	64.0	65.3	95.0	78
E	14.5	.47	3.2	12.0	65.0	68.9	90.0	83
Mean	13.7	.52	3.20	9.2	64.2	65.8	90.2	75.5
S.D. [1]	.80	.10	.39	1.82	1.03	2.00	6.2	7.07
C.V. [2]	.06	.20	.12	.20	.02	.03	.07	.09
Bakery flours—20 samples								
F	11.3	.44	2.4	3.5	61.0	60.9	71.4	47
G	11.4	.42	2.8	5.5	62.0	63.9	78.6	65
H	12.4	.44	2.2	6.0	63.0	64.9	85.4	65
I	13.5	.45	3.2	9.0	64.0	68.5	90.0	82
J	12.7	.47	3.5	6.0	63.0	64.9	81.9	79
Mean	11.9	.47	3.20	5.6	62.2	63.0	80.7	68.5
S.D. [1]	.94	.04	.72	2.08	1.24	2.18	8.58	12.90
C.V. [2]	.08	.08	.22	.37	.02	.03	.11	.19
Topping flour—11 samples								
K	10.1	.40	2.5	4.5	60.0	62.2	74.9	53
L	10.5	.45	2.7	3.5	60.0	61.2	70.7	45
M	11.4	.48	2.4	5.0	62.0	59.2	73.9	58
N	11.6	.45	3.0	6.5	62.0	63.6	84.8	69
O	12.7	.44	3.2	8.0	61.0	64.7	88.3	82
Mean	11.4	.44	2.90	6.0	62.5	62.3	79.4	63.5
S.D. [1]	1.07	.04	.53	2.11	1.22	2.45	7.25	12.69
C.V. [2]	.09	.10	.18	.35	.02	.04	.09	.20
Family flour—19 samples								
P	9.1	.44	2.0	2.5	59.0	54.9	50.0	38
Q	9.8	.48	2.8	4.5	60.0	58.7	69.2	49
R	11.3	.47	3.0	5.0	63.0	59.5	72.9	52
S	11.0	.43	2.8	6.5	62.0	63.4	85.5	58
T	10.9	.47	3.3	14.0	61.0	59.7	80.6	82
Mean	11.1	.42	2.87	7.1	62.0	62.0	76.4	61.5
S.D. [1]	1.33	.11	.92	3.46	1.73	3.43	10.90	14.80
C.V. [2]	.12	.25	.32	.49	.03	.06	.14	.24

[1] Standard deviation.

[2] Coefficient of variation.

The values in Table I indicate that the protein content had a range of 2% or more in each group. The family flours had slightly greater variation than the hearth, bakery, and topping flours. The hearth flours had the highest protein content with the bakery and topping flours intermediate and the family flours lowest.

Flour absorption is positively correlated with the protein content. Thus, the absorptions for the hearth flours were greater than for the

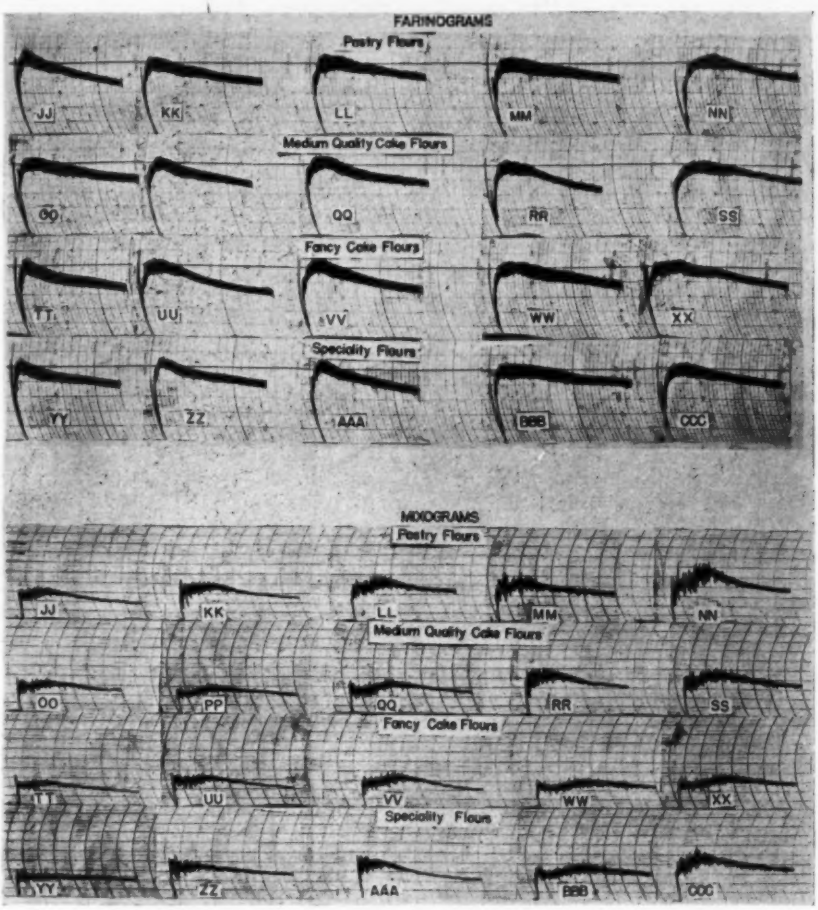

Fig. 2. Farinograms and mixograms for pastry, cake, and specialty flour.

bakery, topping, or family flours. The absorptions determined by the farinograph showed greater variation than those employed in making the mixograms.

There was only slight variation in the mean mixogram mixing requirement of the four flour groups. The farinograms exhibited greater variation between groups. The hearth group had the longest

TABLE II

SUMMARY OF DATA ON PASTRY TYPE FLOURS

Fig. 2 Letter	Protein	Ash	Mixogram mixing time	Farinogram mixing time	Mixogram absorption	Farinograph absorption	Mixogram area	Farinograph valorimeter reading
	%	%	*min.*	*min.*	%	%	*cm* [2]	*unit*
Pastry flours—13 samples								
JJ	9.1	.41	2.0	2.0	59.0	56.0	49.6	36
KK	7.4	.45	2.2	2.0	56.0	53.9	40.4	40
LL	9.7	.42	2.3	2.5	59.0	55.9	53.7	47
MM	10.0	.54	2.0	1.5	60.0	56.4	58.9	49
NN	9.3	.40	2.5	5.0	59.0	62.3	71.4	54
Mean	9.3	.43	2.2	2.6	58.8	56.7	51.1	43.8
S.D. [1]	1.33	.05	.36	1.43	1.73	2.53	10.08	8.06
C.V. [2]	.14	.11	.16	.55	.03	.05	.20	.18
Medium quality cake flours—13 samples								
OO	8.8	.35	2.3	3.0	58.0	55.1	38.5	45
PP	8.5	.33	2.3	2.0	58.0	52.9	39.4	36
QQ	9.0	.70	2.8	2.0	58.0	52.4	47.7	41
RR	8.9	.43	2.0	3.0	58.0	59.3	51.5	38
SS	10.4	.36	2.8	4.5	60.0	58.0	80.0	53
Mean	8.8	.41	2.3	2.9	57.9	55.2	50.3	41.8
S.D. [1]	.62	.09	.40	.65	.96	3.49	10.39	4.42
C.V. [2]	.07	.22	.18	.22	.02	.06	.21	.11
Fancy cake flours—15 samples								
TT	7.8	.33	2.0	2.0	57.0	51.6	33.7	36
UU	7.9	.36	2.0	3.0	57.0	57.0	40.0	40
VV	8.5	.35	2.2	3.0	58.0	53.6	45.8	43
WW	8.3	.35	3.0	3.5	56.0	53.8	40.0	47
XX	8.8	.39	3.3	4.5	58.0	56.2	49.9	52
Mean	8.6	.37	2.4	3.1	57.8	54.8	45.7	42.1
S.D. [1]	.99	.03	.46	1.86	1.41	2.27	9.98	13.37
C.V. [2]	.12	.08	.19	.60	.02	.04	.22	.32
Specialty flours—7 samples								
YY	7.8	.39	1.2	1.0	57.0	53.5	37.2	38
ZZ	7.0	.44	2.0	1.5	56.0	53.7	48.0	40
AAA	9.2	.44	1.7	1.5	59.0	54.3	52.0	35
BBB	9.8	.42	3.5	4.0	59.0	54.9	48.2	52
CCC	10.4	.43	2.0	3.5	60.0	58.2	53.0	50
Mean	9.6	.41	2.2	2.1	58.1	54.6	47.4	41.6
S.D. [1]	2.40	.03	.79	1.17	1.22	1.63	8.91	7.02
C.V. [2]	.25	.06	.36	.56	.02	.03	.19	.17

[1] Standard deviation. [2] Coefficient of variation.

mixing requirement and the family, topping, and bakery had shorter mixing requirements. The family flour had the greatest variation in mixing requirement.

The mixogram area and farinogram valorimeter reading were largest for the hearth bread flours, intermediate for the bakery and topping flours, and lowest for the family flours. The within group variation was greatest for the family, intermediate for the bakery and topping, and smallest for the hearth flours. While the four different flour groups are not sharply differentiated by mixogram area and

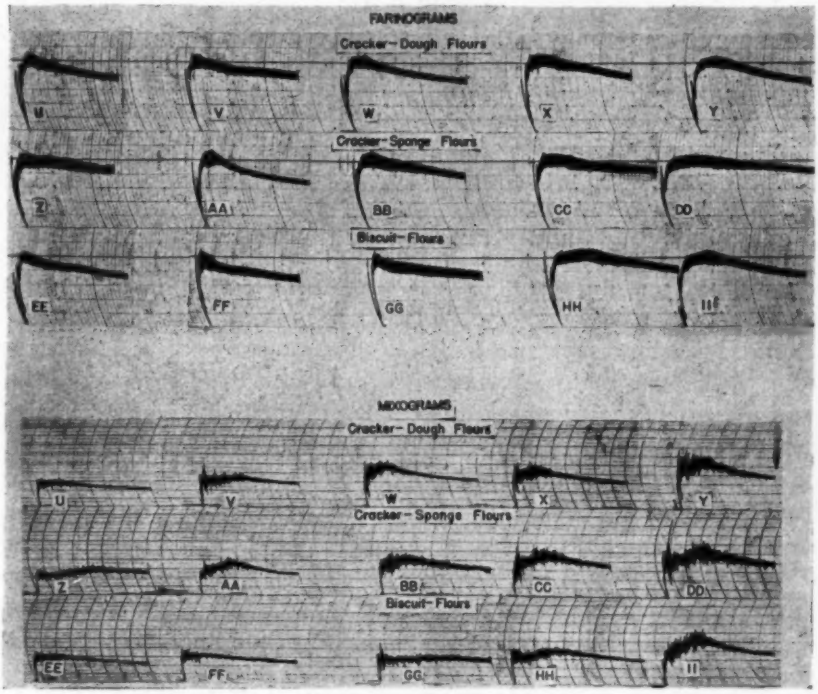

Fig. 3. Farinograms and mixograms for cracker and biscuit flour.

valorimeter reading, the means indicate that hearth flours are the strongest, followed in order by bakery, topping, and family flours.

Pastry, Cake, and Specialty Flours. Farinograms and mixograms of flours used for pastry, cake, and several specialties such as doughnut and pie crusts are shown in Figure 2, and the corresponding data are given in Table II. The arrangement of curves and data are the same as used in Figure 1 and Table I. The farinograms and mixograms in Figure 2 are, in general, strikingly different from those in Figure 1. As compared with the bread flours, the pastry and specialty flours are characterized mainly by shorter mixing requirement, greater sensitiv-

ity to overmixing, lower protein and ash content, lower absorption, and smaller area and valorimeter reading.

The differences between the four groups of pastry flours (Figure 2, Table II) were small and the variations within each group relatively large, thus making this differentiation into groups of doubtful value. The fancy cake flours have the lowest protein and ash contents, followed by the medium quality cake, pastry, and specialty flours. The specialty flours had the greatest variation in protein content, being followed by pastry, fancy cake, and medium quality cake flours. All

TABLE III

SUMMARY OF DATA ON CRACKER TYPE FLOURS

Fig. 3 Letter	Protein	Ash	Mixogram mixing time	Farinogram mixing time	Mixogram absorption	Farinograph absorption	Mixogram area	Farinograph valorimeter reading
	%	%	*min.*	*min.*	%	%	*cm* ^{2}	*unit*
Cracker dough flours—6 samples								
U	9.1	.41	2.1	2.0	59.0	54.5	38.1	40
V	7.4	.38	3.0	1.5	56.0	53.0	41.3	43
W	9.0	.48	2.0	3.0	58.0	59.9	65.0	45
X	9.9	.43	1.8	3.0	59.0	57.4	59.8	47
Y	9.9	.52	2.0	4.5	59.0	61.9	65.3	50
Mean	9.0	.44	2.5	2.8	58.2	56.2	51.2	46.7
S.D. [1]	.93	.05	.85	1.02	1.18	4.45	13.51	5.31
C.V. [2]	.10	.11	.34	.36	.02	.08	.26	.11
Cracker sponge flours—8 samples								
Z	9.3	.39	4.0	2.0	59.0	55.3	42.9	49
AA	9.9	.40	2.5	2.0	50.0	56.9	42.6	39
BB	10.0	.48	2.0	2.5	60.0	57.7	52.4	45
CC	9.9	.40	2.5	3.0	59.0	55.6	60.0	52
DD	10.6	.40	3.2	5.0	60.0	57.7	72.9	62
Mean	9.7	.44	2.4	2.9	59.0	58.3	55.1	47.4
S.D. [1]	1.11	.05	.81	1.09	1.31	3.27	17.29	7.48
C.V. [2]	.11	.11	.34	.38	.02	.06	.31	.16
Biscuit flours—7 samples								
EE	8.2	.44	1.2	2.0	58.0	54.5	42.9	39
FF	7.1	.41	1.9	1.0	56.0	52.2	45.9	32
GG	7.0	.37	3.8	1.0	56.0	51.1	47.1	36
HH	9.4	.36	3.8	6.0	59.0	58.8	60.2	60
II	9.5	.40	2.8	5.0	59.0	61.7	88.6	48
Mean	8.0	.39	2.6	2.4	57.6	54.4	53.9	41.3
S.D. [1]	1.08	.03	1.00	2.15	1.53	4.51	16.31	9.74
C.V. [2]	.14	.07	.39	.90	.03	.08	.30	.24

[1] Standard deviation. [2] Coefficient of variation.

mixing requirements were short and little difference existed between the four groups. The mixogram absorptions were similar for each group, but the farinogram absorptions for the pastry flours were slightly higher than for the other flours. Only small differences existed between the four groups in the mixogram area and valorimeter reading. The valorimeter readings for the fancy cake flours showed the largest variations.

Cracker and Biscuit Type Flours. Farinograms and mixograms obtained from cracker dough, cracker sponge, and biscuit type flours are shown in Figure 3 and the corresponding data are presented in Table III.

Many of the farinogram and mixogram patterns of the cracker and biscuit type flours were similar to the pastry type flours (Figure 2). The cracker and biscuit flours, in general, exhibited wider variations in mixogram area and valorimeter reading than the pastry type flours. The protein contents and absorptions were about equal for both types, but the cracker flours had slightly larger mixogram area and valorimeter reading. In contrast with the bread type flours, the cracker and biscuit flours had short mixing requirement, lower protein and absorption, and smaller area and valorimeter readings. The ash contents of the cracker flours were about equal to the bread flours.

The differences in curve patterns between groups in Figure 3 and Table III are probably less than the differences within each group. There is no sharp line of demarcation between groups, but the statistical means in Table III show that certain differences exist between groups. The biscuit flours have less protein and ash than the cracker sponge and cracker dough flours. This is likewise reflected in the lower absorption of the biscuit flours. The biscuit flours on the average gave slightly smaller valorimeter readings than the cracker dough and cracker sponge flours.

Conclusions

The farinograms and mixograms presented in Figures 1, 2, and 3 show a wide range in characteristics within groups as well as between groups. This makes it appear that flours used for the same purposes may have widely different mixing characteristics. It is not likely, however, that all flours within a group are equally satisfactory for the purpose intended. On the other hand, it is likely that the many different flours would require different treatment by the baker in order to obtain satisfactory products.

The protein content appears to be one of the most important characteristics determining the farinogram and mixogram patterns. As the protein content increased, the absorption, mixing requirements, mixogram heights, band width, and area and farinogram valorimeter

reading also increased. The bread flours which were highest in protein content produced patterns which were distinctly different from those for flours of lower protein content. The bread types are characterized particularly by a long mixing requirement or dough development period. Pastry and cracker type flours, for which low protein is desired, exhibited a wide range of patterns that was generally different from the bread types.

The mixogram area was positively correlated with the valorimeter reading. Both characteristics were positively correlated with protein content. Since protein content, in general, is a factor mainly responsible for strength in commercial flours, it is likely that either the area or the valorimeter reading would give indications of the strength. Strength is desirable for bread flours; while for pastry and cracker types of baking, less strength is desirable. Either the area or valorimeter reading gives figures which indicate in a general way the strength desired for widely different types of baking.

Summary

Farinograms and mixograms were made from 132 commercial flours that were classified into 11 groups on basis of intended usage. The 11 groups were assembled into three categories, namely, bread, pastry, and cracker and biscuit. Protein, ash content, mixing requirements, absorptions, and mixogram areas and farinogram valorimeter readings were employed to study the relationship between curve patterns and intended uses.

Bread flours were characterized by higher protein, higher absorption, longer mixing requirement, and larger mixogram area and farinogram valorimeter readings than the pastry and cracker and biscuit flours. The mixograms of the bread flours had greater height and band width than those made from pastry or cracker and biscuit flours. Cracker and biscuit flours produced curves very similar to the pastry flours and had slightly larger area and valorimeter reading than the pastry flours.

In the bread flours small differences existed between the hearth, bakery, topping, and family groups. In general, as much variation was observed within each group as between the various groups. The hearth group had the highest protein and ash content, longest mixing requirement, highest absorption, and largest mixogram area and valorimeter readings. In these characteristics the hearth group was followed in descending order by bakery, topping, and family flours.

Pastry flours showed little difference, if any, in curve patterns between pastry, medium quality cake, fancy quality cake, and specialty pie crust and doughnut flour groups.

Cracker sponge and cracker dough flours produced patterns that

were but slightly different from the biscuit flours, although variation in each group was large. Biscuit flours had shorter mixing requirements, lower absorption, and smaller valorimeter readings than the cracker flours. Little difference was discernible between cracker dough and cracker sponge flours.

Farinograms and mixograms showed a wide range in characteristics within groups as well as between groups. Flours used for the same purpose may have widely different mixing characteristics. It is likely that flours used for the same purpose are satisfactory only if the baker gives each flour different treatment.

Protein content appeared to be one of the most important factors in determining the farinogram and mixogram patterns. Because protein content is mainly responsible for strength in flours and, since mixogram area and valorimeter readings are correlated with protein content, these curve measurements serve to aid in flour classification when fine distinctions between groups or types are not required.

Acknowledgment

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EXTENSOGRAPH STUDIES OF COMMERCIAL FLOURS AND THEIR RELATION TO CERTAIN OTHER PHYSICAL DOUGH TESTS [1]

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Interesting relationships between physical dough mixing properties and other quality factors have been disclosed by numerous investigators. However, it is generally believed that testing of doughs under continuous mixing fails to give a complete insight into the effects of such factors as fermentation, mechanical or chemical treatment, on extensibility and related physical dough properties.

Bailey (1940) has presented a comprehensive survey of various physical dough tests, including those made after rest periods. Munz and Brabender (1940) gave a description of the extensograph, an instrument designed to test extensibility and resistance to extension after various periods of rest. They indicate that a study of the rate, direction, and magnitude of change in extensibility and resistance to extension after a time of rest would be a valuable aid in classifying flours for specific uses.

The same authors (Munz and Brabender, 1941) concluded that a combination of farinogram and extensogram data serves to classify soft wheat flours as to their adaptability for specific uses. Soft wheat flours gave extensograms of small area, while the hard wheat flours gave curves of large area and exhibited greater response to rest periods. Pastry and cooky flours exhibited little changes in any extensogram dimension with increasing rest time. Fancy cake flour doughs possessed small extensibility and relatively large resistance to extension as indicated by a large F/E ratio. Cracker flour doughs were characterized by larger extensogram dimensions than the pastry, cooky, or cake flour doughs.

Munz and Brabender (1940a and 1941) also demonstrated positive relationships between extensogram dimensions, protein content, and various farinogram measurements. Aitken, Fisher, and Anderson (1944) corroborated the evidence of Munz and Brabender (1941). Significant positive relationships between extensogram length, height, and protein content were found. Farinogram development time was positively correlated with extensogram height.

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[1] Contribution No. 126, Department of Milling Industry.

The purpose of this investigation was to make a detailed study of the value of extensograms for classifying flours for specific uses. Since data from farinograms and mixograms for the same groups of flours were available, the relationships between extensogram, farinogram, and mixogram characteristics were studied.

Materials and Methods

One hundred and thirty flours obtained from flour mills and bakeries located in various parts of the United States and Canada were used. The following groups and number of flours were obtained: hearth, 13; bakery, 20; topping, 11; family, 19; pastry, 13; medium quality cake, 13; fancy cake, 15; specialty, 8; cracker dough, 6; cracker sponge, 6; and biscuit, 6. A further description of these samples is given by Johnson, Shellenberger, and Swanson (1946).

Extensograms were made according to the instructions supplied by the Brabender Corporation for the use of their instrument. All doughs were mixed in the large farinograph bowl, freed of oxides, to a 500 unit consistency. Flour (300 g, 14% moisture basis), water, and 2% salt were mixed for one minute, followed with a 5 minute rest period and then mixed to the point of minimum mobility. The dough was then scaled to 150 g, rounded up by 20 revolutions of the rounder, molded and placed in the thermostat of the extensograph at 30°C for 45 minutes, and an extensogram made. Repeat tests on the same dough piece were made at 45 minute intervals. For purposes of presentation and discussion, however, only the extensograms made after 45, 135, and 225 minute rest periods were chosen. The extensibility or length (E) was expressed in number of centimeters that the dough was stretched before rupturing. Resistance to extension or height (H) was measured in chart units and represents the maximum force applied to the dough before rupturing. The area was measured with a planimeter and expressed in square centimeters.

The choice of extensograms to represent the various flour groups was made on the basis of maximal, minimal, and "typical mean" values of the curve area. The figures for extensibility and resistance to extension are the values that correspond to the curve chosen on basis of area and may not necessarily correspond to the actual extremes and means of extensibility and resistance to extension. In addition to the curves chosen to represent the flours, the means, standard deviations, and coefficients of variability were calculated from data representing all the flours within given groups. The "typical mean" may not agree exactly with the statistical mean since the former was chosen on the basis of the curve area that approximated the statistical mean.

The farinogram and mixogram data employed to study the relationship of the extensogram characteristics to those of the farinograms and mixograms were, in part, obtained from a previous study reported by Johnson, Shellenberger, and Swanson (1946). In addition to the farinogram and mixogram characteristics discussed by these authors, mixogram heights and protein contents were included in the present study. The maximum value of extensibility, resistance to extension, and area of the extensograms for each flour were correlated with the farinogram, mixogram, and protein content data. The maximum values representing extensibility, resistance to extension, and extensogram area were taken from the extensograms after various rest periods, and thus no uniform rest period is indicated for all extensogram properties nor for the different flours.

Results and Discussion

Extensograms of Flours Intended for Specific Use. Extensograms for flours which gave minimal, average, and maximal curve areas within each flour group are presented in Figure 1. The data for these particular flours are given in Tables I, II, and III, together with the means, standard deviations, and coefficients of variability computed from the results for all flours within each group. The data for the 11 flour groups were assembled into three main categories or types: (1) bread flours, (2) cracker and biscuit flours, and (3) pastry flours.

No sharp line of demarcation may be drawn between the three main types of flours (Figure 1). The extensograms which exhibited maximal areas among the cracker and biscuit flours (curves O, R, and U), are not greatly different from the mean curves E, H, and K for the bread flours. Likewise, the curves of minimum area for the bread flours (curves D, G, and J) are not greatly different from the mean curves (N and Q) for the cracker and biscuit types or the maximal curves (X and Gg) of the pastry flours. However, certain numerical average differences between the three types may be observed. The bread flours (Table I), on the average, contained more protein and produced doughs of greater extensibility, greater resistance to extension and greater extensogram area than either the cracker and biscuit or pastry flour doughs. The cracker and biscuit types (Table II) on the average possessed as much protein as the pastry type flours (Table III). However, the cracker and biscuit doughs exhibited slightly greater extensibility, greater resistance to extension, and greater extensogram area than did the pastry flour doughs. The bread flour doughs exhibited greater response to rest periods than did the other two types. The low extremes illustrated by curves M, P, S, V, and Ee exhibited little response to rest periods compared to most other curves, for example A, B, C, or I.

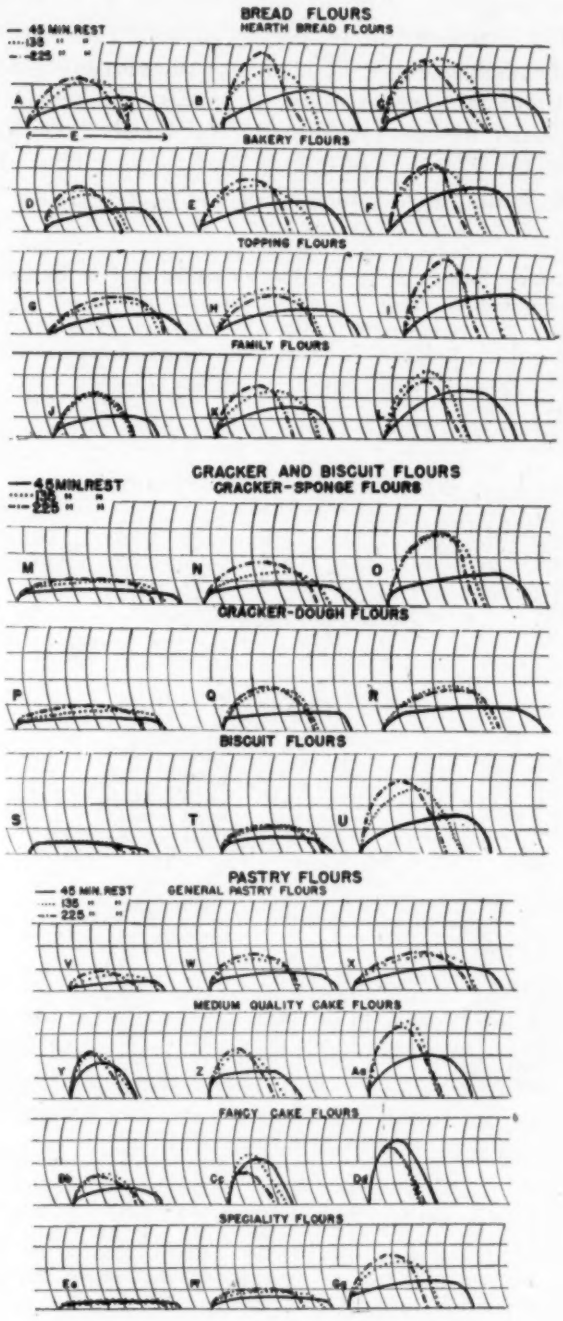

Fig. 1. Extensograms for the various types of flours.

The various groups of bread flours (Figure 1, Table I) tended to have different extensogram properties although no distinct dividing line existed between groups. The mean values for protein and extensogram height and area tended to be highest for the hearth flours, being followed in order by bakery, topping, and family flours. The higher extremes (curves F and I) of bakery and topping flours have properties that are similar to the means for the hearth group. The coeffi-

TABLE I

SUMMARY OF PROTEIN AND EXTENSOGRAM DATA FOR BREAD FLOURS

	Protein	Length			Height			Area		
		45 min.	135 min.	225 min.	45 min.	135 min.	225 min.	45 min.	135 min.	225 min.
	%	*cm*	*cm*	*cm*	*units*	*units*	*units*	cm^2	cm^2	cm^2
HEARTH BREAD FLOURS—13 SAMPLES										
Minimum[1]	11.0	20	15	15	310	470	480	99	105	108
Mean	13.3	26	19	14	320	560	720	113	127	118
Maximum	14.6	27	18	17	345	660	660	125	159	131
Mean[2]	13.7	24	16	15	384	601	649	124	123	119
Std. Dev.	.80	3.7	3.0	3.5	90.9	93.8	74.2	13.9	22.3	26.1
C.V.[3]	.06	.16	.19	.23	.24	.16	.11	.11	.18	.22
BAKERY FLOURS—20 SAMPLES										
Minimum	10.5	26	15	14	235	380	450	62	71	80
Mean	12.4	26	20	17	290	440	515	94	114	106
Maximum	12.6	27	23	28	460	640	680	134	134	114
Mean	11.9	23	18	15	361	563	615	102	114	106
Std. Dev.	.94	2.9	4.9	4.1	68.0	102.2	108.9	18.5	25.9	15.7
C.V.	.08	.12	.27	.27	.19	.17	.18	.18	.23	.15
TOPPING OR DOUGHING FLOURS—11 SAMPLES										
Minimum	10.7	24	21	20	108	365	315	62	98	80
Mean	10.6	26	18	18	260	450	380	85	106	89
Maximum	13.8	25	18	13	425	622	752	138	137	122
Mean	11.4	23	17	15	299	517	515	89	104	94
Std. Dev.	1.07	2.4	2.0	2.4	95.3	115.8	123.1	25.5	13.0	17.8
C.V.	.09	.11	.12	.16	.32	.22	.24	.29	.13	.19
FAMILY FLOURS—19 SAMPLES										
Minimum	10.1	19	15	15	210	415	450	56	75	77
Mean	9.8	21	19	14	305	445	500	87	108	88
Maximum	10.9	23	15	14	490	645	590	146	121	90
Mean	11.1	21	16	15	315	506	518	89	106	93
Std. Dev.	1.33	2.3	2.2	2.7	71.9	111.2	118.8	21.9	21.3	19.8
C.V.	.12	.11	.14	.18	.23	.22	.23	.25	.20	.21

[1] Minimum, mean, and maximum refer to extensograms in Figure 1 for each group.
[2] Statistical mean for each group.
[3] Coefficient of variability for each group.

TABLE II

SUMMARY OF PROTEIN AND EXTENSOGRAM DATA FOR CRACKER AND BISCUIT FLOURS

	Protein	Length			Height			Area		
		45 min.	135 min.	225 min.	45 min.	135 min.	225 min.	45 min.	135 min.	225 min.
	%	*cm*	*cm*	*cm*	*units*	*units*	*units*	*cm²*	*cm²*	*cm²*
CRACKER SPONGE FLOUR—6 SAMPLES										
Minimum[1]	10.7	24	21	20	120	180	200	41	52	54
Mean	9.4	22	18	16	170	270	340	55	63	73
Maximum	10.6	21	14	13	270	560	580	78	90	98
Mean[2]	9.7	24	17	18	158	250	280	56	69	72
Std. Dev.	1.11	1.4	3.2	3.7	47.8	127.1	120.5	12.3	4.4	13.8
C.V.[3]	.11	.06	.19	.21	.30	.51	.43	.22	.06	.19
CRACKER DOUGH FLOUR—6 SAMPLES										
Minimum	9.0	21	21	18	80	150	180	30	44	48
Mean	9.9	19	13	13	125	320	340	40	58	57
Maximum	9.9	24	16	17	200	360	330	68	73	68
Mean	9.0	20	17	16	159	228	258	49	52	52
Std. Dev.	.93	4.8	3.8	2.9	86.2	106.2	91.8	19.2	13.9	10.4
C.V.	.10	.24	.22	.18	.54	.47	.36	.39	.27	.20
BISCUIT FLOUR—6 SAMPLES										
Minimum	7.1	17	15	13	90	90	100	24	20	18
Mean	7.0	15	15	14	140	220	240	32	47	45
Maximum	9.4	19	16	15	310	520	590	79	98	99
Mean	8.0	16	16	15	251	283	324	44	54	66
Std. Dev.	1.08	3.3	2.3	1.5	284	221	230.8	28.5	30.0	45.4
C.V.	.14	.21	.15	.10	1.13	.78	.71	.65	.55	.69

[1] Minimum, mean, and maximum refer to extensograms in Figure 1 for each group.
[2] Statistical mean for each group.
[3] Coefficient of variability for each group.

cient of variability (Table I) for extensibility increased as the length of the curves decreased. This may be caused by the fact that the differences in the flours within a group tended to be accentuated as the rest periods became longer. Most flours in each group tended to produce curves with maximum area at 135 minutes of rest, while with each successive 45 minute rest period, the dough became less extensible and required greater force to be extended.

The cracker dough and sponge flours (Figure 1, Table II) produced doughs with about the same extensibility, but the cracker sponge doughs tended to show more response to 225 minutes of rest than did the cracker dough flour. The biscuit flours produced extensograms of less length and of smaller area than the cracker sponge and dough flours. The low extremes (curves M, P, and S, Figure 1) exhibited

little response to rest periods compared to the high extremes (curves O, R, and U). These results appear to corroborate the conclusions of Munz and Brabender (1941).

The general pastry flour group (Figure 1, Table III) appears to have characteristics different from those of the cake or specialty flour groups. The general pastry flour doughs are characterized by greater

TABLE III

SUMMARY OF PROTEIN AND EXTENSOGRAM DATA FOR PASTRY, CAKE FLOURS, AND SPECIALTY FLOURS

	Protein	Length			Height			Area		
		45 min.	135 min.	225 min.	45 min.	135 min.	225 min.	45 min.	135 min.	225 min.
	%	*cm*	*cm*	*cm*	*units*	*units*	*units*	cm^2	cm^2	cm^2
GENERAL PASTRY FLOUR—13 SAMPLES										
Minimum[1]	7.1	17	15	11	100	170	210	24	37	31
Mean	9.8	21	15	16	170	300	340	55	65	65
Maximum	12.6	25	19	19	200	300	330	69	77	79
Mean[2]	9.3	18	14	14	214	337	352	50	58	60
Std. Dev.	1.33	4.2	4.1	3.8	85.3	103.7	81.5	13.4	13.3	15.0
C.V.[3]	.14	.24	.29	.28	.40	.31	.23	.27	.23	.25
MEDIUM QUALITY CAKE FLOUR—13 SAMPLES										
Minimum	8.5	12	11	9	340	430	440	56	57	48
Mean	8.6	15	12	11	280	440	480	59	69	63
Maximum	10.4	16	11	12	390	750	650	90	101	94
Mean	8.8	14	11	10	339	517	502	67	69	61
Std. Dev.	.62	2.0	1.3	1.4	66.1	114.4	78.8	13.6	19.5	16.3
C.V.	.07	.14	.12	.14	.20	.22	.16	.20	.28	.27
FANCY CAKE FLOUR—15 SAMPLES										
Minimum	7.8	16	11	11	160	300	280	35	42	42
Mean	8.4	11	10	8	425	480	320	59	56	31
Maximum	8.9	11	9	9	590	520	500	80	56	57
Mean	8.6	13	10	10	569	450	391	60	52	47
Std. Dev.	.99	1.7	1.6	1.3	112.9	111.2	72.2	13.5	12.1	93.2
C.V.	.12	.13	.17	.13	.20	.25	.18	.22	.23	.20
SPECIALTY FLOURS—8 SAMPLES										
Minimum	9.2	23	20	19	100	100	70	23	28	24
Mean	8.9	21	19	16	110	170	200	33	48	47
Maximum	9.8	21	15	14	260	460	500	77	90	89
Mean	9.6	20	17	16	138	194	213	40	47	44
Std. Dev.	2.40	2.6	2.6	2.0	62.2	125.0	141.4	20	22.8	22
C.V.	.25	.13	.15	.13	.45	.64	.66	.50	.49	.50

[1] Minimum, mean, and maximum refer to extensograms in Figure 1 for each group.
[2] Statistical mean for each group.
[3] Coefficient of variability for each group.

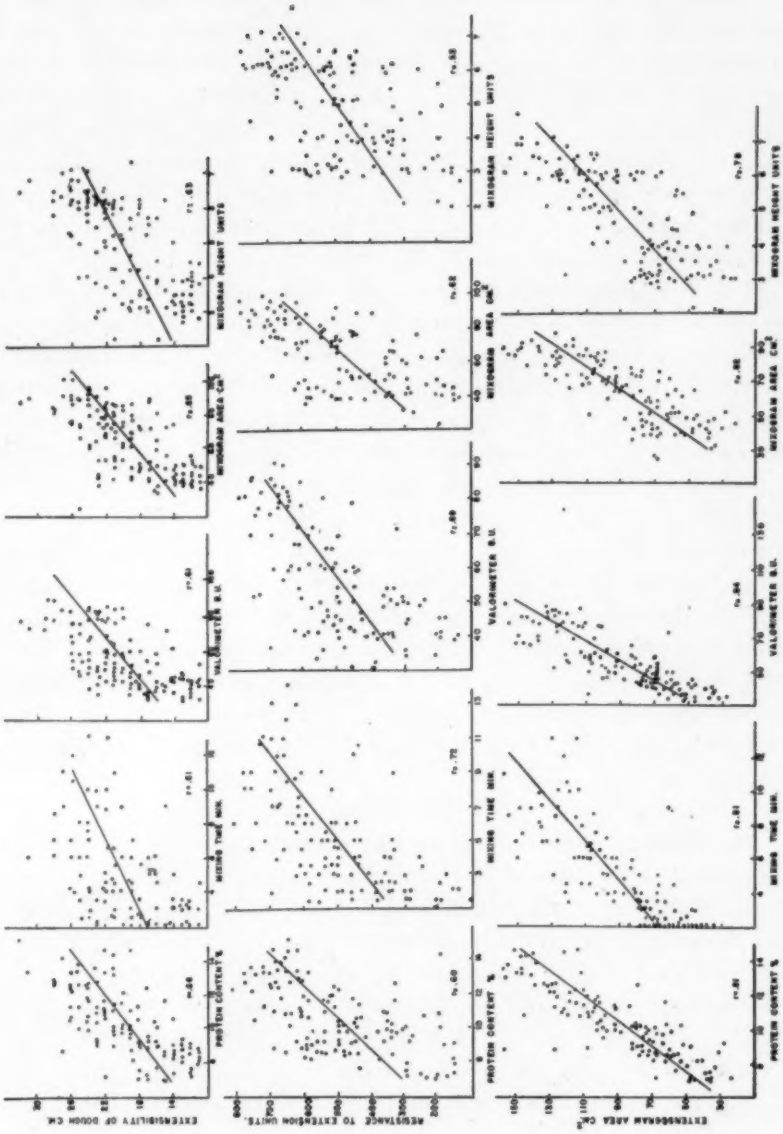

Fig. 2. The relationship of dough extensibility, resistance to extension, and extensogram area to protein content, farinogram mixing time, valorimeter value, mixogram area, and height. The correlation coefficient is included for each relationship.

extensibility and less resistance to extension than the cake flour doughs. The latter exhibit small extensibility in comparison to the resistance to extension. The specialty flour doughs had relatively large extensibility compared to the small resistance to extension. All four groups exhibited wide ranges in all three extensogram properties.

Correlation of Extensogram Properties with Protein Content, Farinogram, and Mixogram Properties. The relationship of dough extensibility, resistance to extension, and extensogram area to protein content, farinogram mixing time, valorimeter value, mixogram area, and height for the 130 samples are shown by scatter diagrams and regression lines in Figure 2. Also, included in the diagrams are the correlation coefficients for each relationship studied.

All the variables studied were positively correlated but the correlations are not of sufficient magnitude to have practical significance for purposes of prediction. Farinogram valorimeter value and mixogram area, which comprise strength ratings obtained with the two mixing devices, respectively, are associated with extensibility and resistance to extension. Furthermore, extensibility and resistance to extension are positively correlated with protein content, which is generally associated with the strength of a flour for breadmaking purposes. Since area of the extensogram is a function of both extensibility and resistance to extension it is to be expected that the area should be related to the same factors with which extensibility and resistance to extension are correlated. Such proves to be the case as shown by Figure 2. The magnitudes of the correlations between extensogram area and protein content, farinogram and mixogram characteristics, respectively, are slightly greater than those for the single extensogram factors.

Summary

Extensograms were made from 130 commercial flours that had been classified into 11 groups on the basis of intended use. The data for the various groups were assembled under three main categories, namely: bread flours, cracker and biscuit flours, and pastry flours. Measurements of extensibility, resistance to extension, and total area were made from the curves obtained after rest periods of 45, 135, and 225 minutes.

No sharp line of demarcation existed between the three types; since each contained flours exhibiting minimal and maximal curve characteristics which tended to merge into those of other groups.

Extensogram data representing bread type flours including hearth, bakery, topping, and family groups showed greater extensibility, greater resistance to extension, and larger curve area than did those from the biscuit and cracker or pastry flours. Bread flour doughs also

showed greater response to rest periods than did the other types. The biscuit and cracker group tended to have extensogram properties intermediate in magnitude to the bread and pastry flours. The pastry flours produced extensograms of smallest area.

Protein content, extensogram height, and area tended to average highest for the hearth group being followed in order by bakery, topping, and family flours.

Cracker dough and cracker sponge flours exhibited doughs with about equal extensibility but the cracker sponge doughs had greater resistance to extension and larger extensogram area. The extensograph data for the biscuit flours were extremely variable.

Extensograms for the general pastry and specialty flours were much alike. The extensibility of the dough for these two groups was greater than for the cake flours, while the resistance to extension was less. The fancy cake flour doughs possessed the greatest resistance to extension with 45 minutes of rest, while the medium quality cake flour produced doughs that reached their maximum resistance at 135 minutes of rest.

Extensibility, resistance to extension, and extensogram area were each positively correlated with protein content, farinogram mixing time, valorimeter value, mixogram area, and height. Extensogram properties were more highly correlated with protein content than with farinogram or mixogram properties. The correlations between extensogram area and protein content, farinogram, and mixogram characteristics were greater than the correlations between the same mixing factors and the extensibility and the resistance to extension.

Acknowledgment

The authors wish to thank the Brabender Corporation, Rochelle Park, New Jersey, for the loan of the extensograph used in this investigation. Acknowledgment of the technical assistance of August Schmitz from the Brabender Corporation is also given.

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AMYLOGRAPH CURVE CHARACTERISTICS OF VARIOUS TYPES OF COMMERCIAL FLOURS AND THEIR RELATION TO FLOUR MALTOSE AND GASSING POWER VALUES [1]

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Caesar (1932) developed a continuous recording consistometer to measure the changes in consistency of starch dispersions over the entire period of heat gelatinization. Several modifications of this instrument have been developed and used for special studies of the pasting characteristics of starches. Brabender (1937) developed the amylograph which is an improved instrument operating on the same general principle as Caesar's viscosimeter. A full discussion of the working principles of the amylograph is given by Müller (1939). Recently Anker and Geddes (1944) and Brown and Harrel (1944) have thoroughly discussed the operation as well as the theoretical and practical applications of the amylograph to cereal chemical studies.

As pointed out by Anker and Geddes (1944), amylograph curves obtained with wheat flour dispersions represent the result of a number of interacting forces at work as the starch is gelatinized by heat and subjected to amylase action. The peak viscosity of flour pastes depends on several variables such as starch content, particle size, inherent starch characteristics, extent of mechanical injury of the starch, pH, amylase activity, and the rupture of the swollen granules. According to Katz and Hanson (1934), wheat starch swells at 50°C; gelatinization begins at 65°C and is complete at 67.5°C. Amylase action is slight until the gelation point is reached; as the temperature rises above this point, the increase in viscosity due to gelatinization of the starch is opposed by the liquefying action of the amylase and by granule disintegration which results from the internal shearing stress. As more and more of the starch granules become gelatinized and the amylases are heat inactivated, a maximum viscosity is approached at temperatures of 70° to 80°C. The subsequent decline in viscosity is attributed principally to granule disintegration as a result of the internal shearing stress (which becomes pronounced with the increased hydration and closer packing of the granules).

The purpose of this study was to investigate the amylograph curves obtained from a series of widely varying commercial flours manufac-

[1] Contribution No. 127, Department of Milling Industry.

tured for specific uses. An attempt was made to relate the characteristics of the amylograph curve to the specific uses for which the flours were manufactured and also to interpret the curve characteristics in relation to flour maltose value (diastatic activity) and gassing power.

Materials and Methods

One hundred and thirty flours were obtained from various sources. These flours were classified according to the purpose for which they were used commercially. Eleven different groups were formed which included the following flour types: hearth, bakery, topping, family, general pastry, average quality cake, fancy cake, specialty soft wheat, cracker dough, cracker sponge, and biscuit flours. A more complete description of these samples has been given by Johnson, Shellenberger, and Swanson (1946).

Diastatic activity and gas production values of the flours were determined by the methods described in *Cereal Laboratory Methods* (4th ed., 1941). Amylograph curves were obtained for the entire series of samples.

Operation of the Amylograph. A smooth flour-water slurry was formed by beating and shaking 65 g of hard wheat flour or 55 g of soft wheat flour with 450 ml of distilled water at 30°C. A decrease in the amount of soft wheat flour was necessary because the larger percentage of starch in soft wheat would cause the curve peaks of the amylograms to go above the chart paper.

The suspension was poured into the amylograph bowl, the kymograph adjusted to a zero time position, the contact thermometer adjusted to start heating at 30°C, and the instrument started. The temperature of the suspension was increased at a constant rate of approximately 1.5°C per minute for a total of 40 minutes. After 40 minutes a temperature of 90° C was reached and the amylograph was stopped.

Results and Discussion

The highest and lowest amylograph curves within each group for six of the 11 flour types are shown in Figure 1. Similar results were obtained for the five groups not shown. The dotted line indicates the mean value for all curves within each group.

The curves show large differences in height within the same flour group as well as between groups, and also pronounced differences in the number of minutes required to reach the curve peak. The period of time required before the curves began to rise was uniform within each group. Because of the differences in flour concentration between the bread and pastry groups, there is a difference in the number of minutes

required for gelation to start. Any change in the ratio of flour to water will alter the gelation rate and also the curve height.

The amylograph curves used in this study as already stated were obtained from unbuffered water-flour pastes. In some laboratories the pastes are always buffered, but the desirability of buffering may be questioned, especially if the amylograph curves are to be interpreted in terms of starch conversion during the baking of bread. Mangels

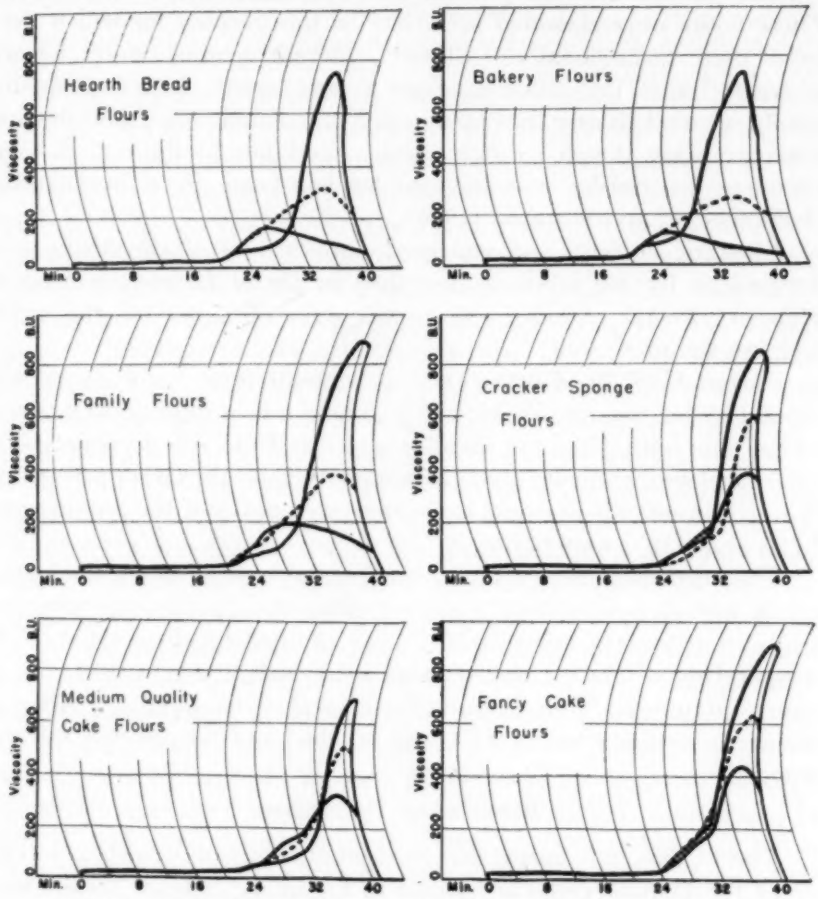

Fig. 1. Highest, lowest, and mean amylograph curves for six types of flours.

and Martin (1935) have shown that buffers will influence the diastatic activity differently depending upon whether the same starch type is cooked or raw. Also, the various buffer types, even at the same hydrogen-ion activity, influence the reaction rate of the amylases. Anker and Geddes (1944) studied the effects of two buffer systems on wheat starch over a pH range from 5.2 to 6.7 and observed that the amylograph curve heights were different. Moreover, the amylases are

known to have different optimum pH values at different temperatures. These considerations complicate the matter of selecting suitable buffer solutions and the proper pH value. Since the change in viscosity recorded by the amylograph is registered only after the starch begins to gel, the pH selected and the buffer adopted should be the ones most effective for the conditions under which the tests are performed. The relationship between amylograph readings and the changes in starch consistency and gas production during the process of baking may be lessened rather than strengthened by the buffering of the amylograph suspensions, since buffers are not added to doughs.

In order to compare the effect of buffering on curve characteristics, a diabasic sodium phosphate-citric acid buffer at a pH of 5.35 was used. It was found that the buffered and unbuffered curves were similar in shape and apparently differed only in height.

Figure 2 shows the relationships between (1) diastatic activity (maltose units – mg/10 g) and gassing power (M.M. mercury pressure at five hours); (2) diastatic activity and amylograph curve height (Brabender units); and (3) gassing power and amylograph curve height.

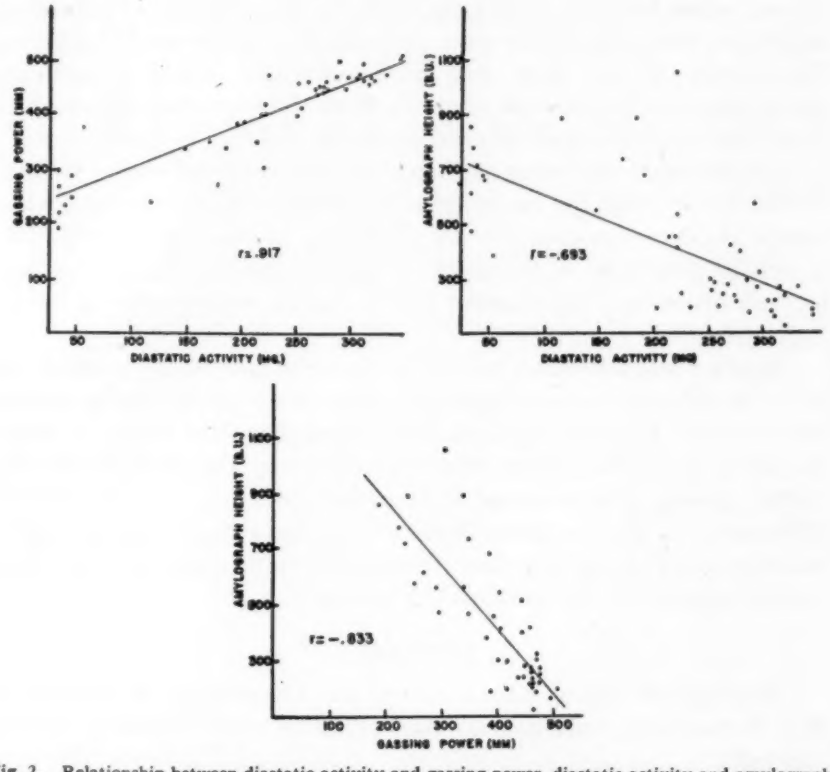

Fig. 2. Relationship between diastatic activity and gassing power, diastatic activity and amylograph curve height, and gassing power and amylograph curve height.

Only the bakery, family, and cracker sponge groups are included in the comparisons since these groups comprise a wide range of flour type. Amylase activity and gassing power are of only minor importance in soft wheat flour.

The relationships between diastatic activity, gassing power, and height of the amylograph curve, as shown in Figure 2, include 45 samples of bakery, family, and cracker sponge type flours. The correlation coefficient for diastatic activity and amylograph curve height was −0.693, for gassing power and amylograph height −0.833, and for diastatic activity and gassing power 0.917.

The relationships among the three measures of amylase activity are interesting. Diastatic activity and gassing power values are obtained from raw starch substrates, one buffered and the other not, while the amylograph readings are made on gelatinized starch at temperatures favoring alpha-amylase activity. The gassing power values involve the use of yeast. Considering the different conditions involved in the measurements, the agreement among the methods is remarkably good.

The variations in the diastatic activity and gassing power values of flours used for the same purpose are well known. The moderate degree of association between these values and the amylograph curve height is indicative that the curves vary considerably within each flour type. The curves did not show any more uniformity within a particular group such as, for example, baker's flour than between flours of entirely different type such as cracker dough and family flour.

Amylographs are being used to some extent by the cereal industry on the theory that the curves give an insight into the changes which starch undergoes within the dough during the baking process. Research to determine more clearly the relationship between the amylograph curves and the changes which doughs undergo during baking is needed.

Another consideration to which thought should be given is the effect of different enzyme types on gelatinized starch during baking. For example, if other amylases than those of malted wheat or barley should be used, the present relationships among the diastatic activity values, gassing power values, and amylograph curves may be altered. Differences in the thermolabilities of amylases from various sources would produce changes in the extent of enzymatic action during baking and be reflected in the amylograph curves.

Summary

Amylograph curves were obtained for 130 samples of commercial flour representing various flour types such as bakery, family, cracker, and cake.

Various types of flour did not give characteristic amylogram curves. Normal curve heights were found to vary nearly as much within a particular flour group as between different flour types.

Amylograph curve heights were negatively correlated with diastatic activity ($r = -0.693$) and with flour gassing power ($r = -0.833$).

Acknowledgment

The authors wish to thank the Brabender Corporation, Rochelle Park, New Jersey, for the loan of the equipment used in this study. They wish also to acknowledge with thanks the technical assistance of August Schmitz.

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WATER RETENTION CAPACITY AS AN INDEX OF THE LOAF VOLUME POTENTIALITIES AND PROTEIN QUALITY OF HARD RED WINTER WHEATS [1]

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(Received for publication October 4, 1945)

A simple and rapid method for estimating the protein quality of hard wheats is urgently needed, especially so in the early phases of the breeding program where only small samples of grain are available. Such a test has been developed in this laboratory. It consists essentially of a determination of the amount of water held by a flour against centrifugal force applied to a water suspension to which lactic acid has been added. The amount of water so held is referred to herein as the water retention capacity. This paper describes the test and presents data obtained in its application to hard winter wheats.

Development and Theory of the Test

Viscosity as a measure of the imbibitional capacity of flour has been advanced by Sharp and Gortner (1923) as an index of flour strength and is extensively used in evaluating soft winter wheats. It has not been applied successfully, however, to varieties of hard wheat. The senior author has determined the viscosity at several protein levels for each of six varieties of hard red winter wheat—Kharkof, Blackhull, Tenmarq, Pawnee, Comanche, and Chiefkan.[3] The relation between viscosity and protein content for three of these varieties is shown in Figure 1A. It appears from these data that viscosity is a linear function of the *quantity of protein* within a variety and that the regression of viscosity on protein content is different for each variety. However, viscosity does not properly evaluate the three varieties since, on the basis of loaf volume–protein relations, Kharkof and Tenmarq have approximately equal protein quality and both are far superior to Chiefkan; whereas, on the basis of the viscosity–protein relations, Tenmarq is definitely inferior to Kharkof and nearly as poor as Chiefkan.

The fact that viscosity does not increase with protein content in the same manner and to the same degree as does loaf volume appears to be due to some physical property of the protein such as the ease with which it is hydrated. It was thought that by taking viscosity and some function of rate of hydration, such as mixing time, into ac-

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Associate Chemist and Junior Chemist, respectively, Division of Cereal Crops and Diseases at the Federal Soft Wheat Laboratory, Wooster, Ohio.

[3] Paper presented at the A.A.C.C. tri-section meeting, Manhattan, Kansas, 1941.

count a better measure of protein quality might be derived. Accordingly, after preliminary trials, viscosity was multiplied by the square root of the mixing requirement of each variety sample as determined by the mixograph. The relation between protein content and viscosity corrected in this manner is shown in Figure 1B. It will be

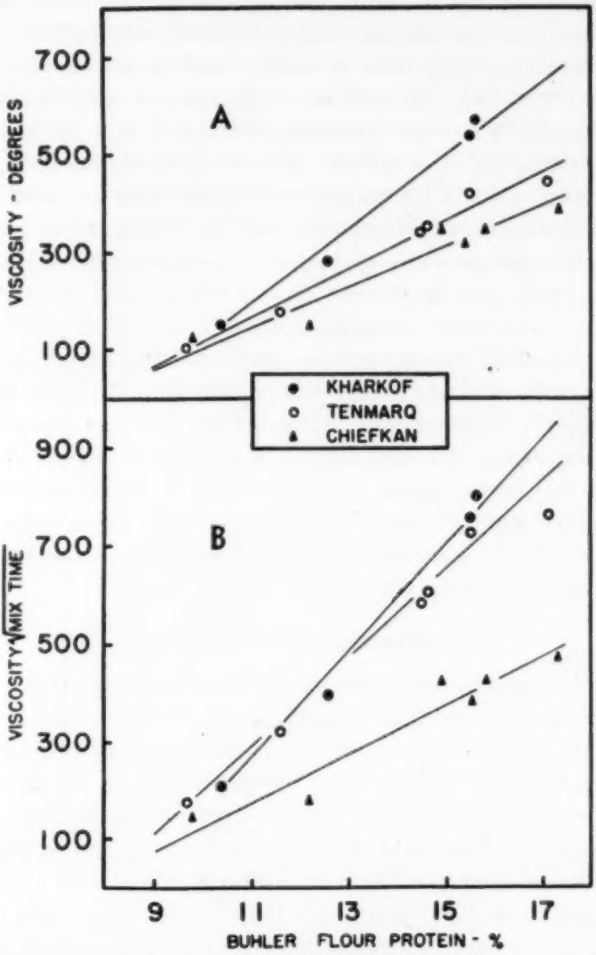

Fig. 1. Relation between protein content and viscosity (above), and viscosity corrected by a hydration factor (below) for Buhler-milled flours of Kharkof, Tenmarq, and Chiefkan.

noted that the regression lines for Kharkof and Tenmarq are now similar, indicating about equal quality, and that both are far above Chiefkan.

Further consideration suggested that the hydration factor possibly was indirectly measuring some physical property such as the tenacity with which the protein holds water in acid media and/or its relative

impermeability under certain conditions of acidity and pressure. It seemed reasonable to expect that an easily hydrated protein meshwork might hold its water of hydration less firmly or might be more permeable than one which is hydrated with more difficulty. There appeared to be a logical analogy between this concept of the capacity of flour proteins to imbibe and to hold water against pressure and certain facts known about gas production and retention in the baking process. For example, in a study with 36 hard winter flours varying in protein content from 9 to 18% for each of six varieties of varying protein quality, proof height in a given time was linear and very highly correlated with protein content ($r = 0.97$). However, when gas production and expansion were greatly increased at the elevated temperature of the baking oven, enormous differences between varieties in gas-retaining ability of the protein were apparent as is indicated by the comparatively low correlation coefficient ($r = 0.81$) for loaf volume and protein content of the same samples. These correlation coefficients indicate that inherent differences in gas-retaining capacity of flours differing in protein quality were not manifested until the protein had been sufficiently inflated and expanded by the gas pressure created in the baking oven. Accordingly, it was conceived that this pressure created in the oven might be paralleled in lactic acid-flour-water suspensions by applying centrifugal force to the water-inflated protein and thereby obtain a measure of water retention capacity for correlating with gas retention capacity.

Material and Methods

The wheat used in this study consisted of representative samples of varieties of hard red winter wheat grown at various locations in the Great Plains area as a part of the coordinated wheat improvement program carried out by the U. S. Department of Agriculture in cooperation with the state agricultural experiment stations. A study was first made of 51 samples of flour milled on the Buhler experimental mill and later of flour produced by grinding wheat in a Hobart Coffee Mill and separating the bran and flour by sifting. The purpose of this latter study was to determine to what extent flour from small samples such as could be ground in a Coffee Mill would provide reliable results. The samples milled on the Buhler mill were from 10 varieties of varying protein quality, Kharkof, Blackhull, Tenmarq, Cheyenne, Nebred, Pawnee, Comanche, Chiefkan, Red Chief, and Wichita, each of which was represented by a low, medium, and high protein level for each of the crop years 1941 and 1942, except for the last two varieties which were from the 1942 crop only. The Hobart flours were milled from the same lots of Chiefkan, Tenmarq, Comanche,

and Cheyenne mentioned above and from three additional lots of Chiefkan and one of each of the other three varieties from the 1943 crop. Thus, 23 of the 29 Hobart flours were from the same samples of wheat milled on the Buhler.

The Buhler mill was operated as described by McCluggage, Anderson, and Larmour (1939). Previous to milling on the Hobart grinder, each 75.0 g wheat sample was passed through the rolls of the Tag-Heppenstall moisture meter. On the basis of the wheat moisture, each Tag-broken sample was tempered to 14% moisture for 24 to 48 hours, after which the Hobart flours were obtained after two grindings on the Hobart Coffee Mill and two siftings. The extremely simple flow sheet showing wire sieves (meshes per inch) and grinder settings (No. 1 and No. 1 − or closed) is shown in Figure 2. The burrs of the

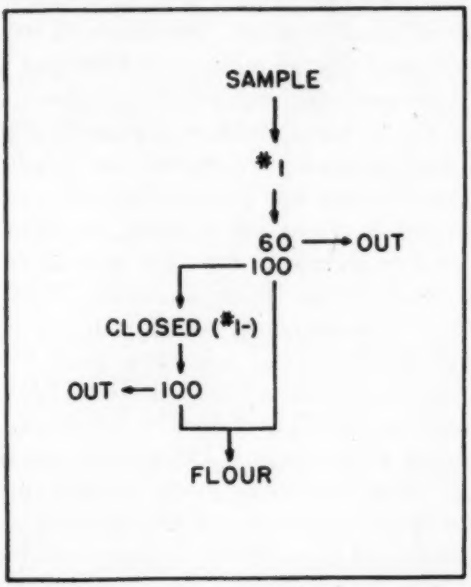

Fig. 2. Flow sheet for Hobart milling procedure showing wire sieves (meshes per inch) and grinder settings (No. 1 and No. 1 −).

grinder touched very faintly at a setting of $1\frac{1}{4}$. The average total time required for grinding, sifting, weighing, and labeling products, calculating yield, and cleaning out the grinder for the next sample averaged 11 to 12 minutes.

The determination of water retention capacity consisted, essentially, of centrifuging a small sample of flour after soaking in water in a test tube to which lactic acid had been added. After centrifuging and pouring off the supernatant liquid, water retention capacity was determined from the increase in weight and expressed as a percentage of the flour.

Preliminary studies showed that the differentiation between varieties increased with the quantity of water used for the soaking and leaching operation previous to adding the lactic acid. In general, however, it is impractical in making numerous determinations to use test tubes larger than about 100 ml. Accordingly, as large a quantity of water was used as was practical with a 100-ml test tube. Studies in varying the quantity of concentrated lactic acid indicated that the ratio of total water to lactic acid should be about 4 to 1 for Hobart flours averaging about 0.90% ash. Lower concentrations decreased differentiation and higher concentrations produced distorted results. With Buhler experimentally milled flours averaging 0.45% ash, however, a satisfactory ratio of total water to concentrated lactic acid was about 67 to 1. The considerably smaller quantity of lactic acid used for Buhler compared to Hobart flour indicates the consideration which must be given to ash content. Studies with the time and speed of centrifuging indicated that 25 minutes at 1800 rpm was satisfactory.

The details of the procedure used were as follows: 5 g of flour was weighed into previously tared, rubber-stoppered, 100-ml pyrex test tubes. Eight determinations were carried out simultaneously. For Hobart flour, 60 ml of water was added to each of the eight tubes one at a time. Immediately after each addition, the stoppered tube with its contents was shaken vigorously for a few seconds to completely wet and suspend the flour. After three additional shakings and a rest period of 4 minutes for sufficiently complete hydration, the stoppers were removed and 20 ml of lactic acid (four parts 85% acid and one part water) was added to each. The eight 20 ml portions were added simultaneously from a unit of eight smaller test tubes. After allowing 1 minute for draining, each was mixed by gently teetering immediately after stoppering. After three more gentle mixings and a rest period of 4 minutes, each stopper was removed and scraped clean at the tube opening. The tubes and contents were then centrifuged for 25 minutes at 1800 rpm in an International Centrifuge, size 2, the supernatant liquid was gently but quickly decanted off, and the tubes allowed to drain into wads of cotton for 5 minutes at an angle of 15° to 20°, after which any liquid adhering at the tube opening was swabbed off with the cotton. The tubes were then stoppered and weighed.

The same procedure was followed for Buhler experimentally milled flours, except that 80 ml of water and 1.5 ml of lactic acid (four parts concentrated and one part water) were used instead of 60 and 20, respectively. Forty-eight determinations can be carried out by one person during an 8-hour day.

The Buhler experimentally milled and unbleached flours were baked using the rich, highly bromated, milk-containing formula in

conjunction with optimum mixing time as described by Finney and Barmore (1945, 1945a). The standard deviation for the average of duplicate determinations made in this laboratory on different days is 10 to 13 cc for loaf volume and 0.75 to 1% for water retention capacity.

Experimental Results

The relation between water retention capacity and loaf volume of bread was first determined for the 51 Buhler experimentally milled flours. The results, with and without lactic acid, are shown graphically in Figure 3. With no lactic acid, there is obviously no relation

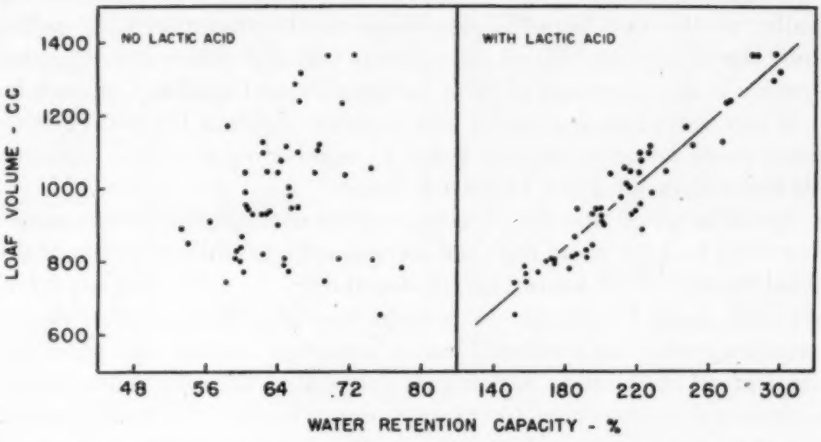

Fig. 3. Relation between loaf volume and water retention capacity with and without lactic acid for 51 Buhler experimentally milled flours representing two crop years and several protein levels for each of 10 hard winter wheat varieties of varying protein quality.

between the quantity of water retained and loaf volume. With lactic acid, however, there is a high positive correlation ($r = 0.95$) and the relation is obviously linear. The standard error of estimate is 55 cc. Considering the large amount of differentiation between samples of over 700 cc, it appears that water retention capacity of the Buhler-milled flours is an excellent index of their loaf volume potentialities.

There were six Chiefkan and three Red Chief samples in the group of 51 Buhler-milled flours. Since the protein quality of these two varieties is generally considered to be distinctly poorer than any of the other eight represented in this study, a pertinent question relates to the relative abilities of water retention capacity and protein content to predict loaf volume. The correlation of protein content with each of the two factors loaf volume and water retention capacity gave coefficients of $r = 0.89$ and $r = 0.84$, respectively, as compared with $r = 0.95$ for water retention capacity and loaf volume. The partial correlation between water retention capacity and loaf volume inde-

pendent of protein content gave a coefficient of $r = 0.82$, indicating that water retention and loaf volume are very highly correlated within groups of samples having the same protein content. The correlation coefficient of $r = 0.95$ for water retention capacity and loaf volume is only slightly less than the multiple correlation coefficient of $R = 0.965$ whereby protein content is taken into account. The standard partial regression coefficients of $\beta = 0.686$ for loaf volume on water retention capacity independent of protein content and of $\beta = 0.314$ for loaf volume on protein content independent of water retention capacity indicate that water retention capacity is more than twice as effective in forecasting loaf volume as is protein content. Nevertheless, the smaller of the two betas is significant far beyond the 0.1% point. Thus, the various statistical calculations indicate that water retention capacity is an expression of both the quality and quantity of protein.

Water retention capacities and protein contents for the Hobart-milled flours are presented in Table I, together with protein contents and loaf volumes for the 23 Buhler flours.

It will be noted that the protein contents of the Hobart flours varied from 0.7% to 2.2% more than the corresponding Buhler experimentally milled flours, except for one which was 0.6% less. The Hobart flours averaged about 1% higher in protein than the Buhler-milled flours. Accordingly the "as received" water retention values were corrected with the aid of the data in Table II (presented later for convenience) to those values expected for Hobart flours having 1% more protein than the corresponding Buhler flours, thereby making the two sets of data comparable. The protein-corrected water retention capacities are included in Table I (last column) and are plotted against the loaf volume for Buhler flours in Figure 4. The correlation coefficient is nearly the same ($r = 0.94$) as was obtained for water retention capacity and loaf volume of the Buhler flours, and the regression is likewise obviously linear. The standard error of estimating loaf volume from Hobart flour water retention capacity is 60 cc. The number of samples is too small to permit positive conclusions, but the results indicate that water retention capacity of Hobart-milled flour is a useful index of loaf volume.

The partial and multiple correlations involving the data for the Hobart flours gave results very similar to those for the Buhler flours, and indicated that water retention capacities for Hobart and loaf volumes for Buhler milled-flours are very highly correlated within groups of samples having the same protein content, that water retention capacity is more than $2\frac{1}{3}$ times as effective in predicting loaf volume as is protein content, and that water retention capacity is an expression of both the quality and quantity of protein.

TABLE I

PROTEIN CONTENT AND PHYSICO-CHEMICAL DATA FOR BUHLER- AND HOBART-MILLED FLOURS OF 29 HARD RED WINTER WHEATS GROWN UNDER DIFFERENT ENVIRONMENTS AND REPRESENTING THREE CROP YEARS AND SEVERAL PROTEIN LEVELS FOR EACH OF FOUR VARIETIES OF VARYING PROTEIN QUALITY

Variety and location	Crop year	Buhler flour[1]		Hobart flour[1]		
		Protein[2]	Loaf volume	Protein[2]	Water retention cap.	
					As rec'd	Adjusted for prot.[3]
		%	%	%	%	%
Tenmarq						
N. Platte, Neb.	1941	13.8	1048	15.2	154.8	151.6
Manhattan, Kans.	1941	12.4	938	13.5	162.5	161.7
Amarillo, Texas	1941	10.0	768	10.9	129.6	130.4
Hays, Kans.	1942	17.1	1235	17.9	197.0	198.6
N. Platte, Neb.	1942	15.6	1123	15.0	150.3	163.1
Woodward, Okla.	1942	10.9	830	11.9	143.2	143.2
Manhattan, Kans.	1943	—	—	11.4	135.9	—
Comanche						
Woodward, Okla.	1941	13.1	928	14.1	148.8	148.8
Amarillo, Texas	1941	11.1	820	12.2	140.8	139.8
Hays, Kans.	1942	18.0	1368	18.7	219.8	222.9
N. Platte, Neb.	1942	14.4	1045	16.2	170.1	161.9
Woodward, Okla.	1942	11.0	825	11.9	147.6	148.6
Manhattan, Kans.	1943	—	—	13.6	170.1	—
Cheyenne						
Sheridan, Wyo.	1941	14.7	927	16.1	166.8	163.5
Lincoln, Neb.	1941	12.8	929	14.0	147.8	146.1
Manhattan, Kans.	1941	11.5	810	12.3	146.0	147.7
Amarillo, Texas	1941	9.5	733	10.4	125.8	126.6
Hays, Kans.	1942	16.9	1120	18.0	195.9	195.1
N. Platte, Neb.	1942	12.9	948	15.1	157.0	147.0
Manhattan, Kans.	1943	—	—	12.6	142.1	—
Chiefkan						
Lawton, Okla.	1941	15.3	921	16.5	148.1	146.8
Lincoln, Neb.	1941	13.2	783	14.2	135.6	135.6
Amarillo, Texas	1941	10.6	655	11.5	116.7	117.3
Hays, Kans.	1942	17.1	1037	17.8	165.8	167.8
N. Platte, Neb.	1942	12.9	748	14.4	129.4	126.1
Woodward, Okla.	1942	11.7	793	12.6	132.4	133.0
Manhattan, Kans.	1943	—	—	13.6	136.7	—
Manhattan, Kans.	1943	—	—	13.2	129.9	—
Manhattan, Kans.	1943	—	—	13.1	133.2	—

[1] The average ash contents for the Buhler and Hobart milled flours were 0.48% and 0.84%, respectively.
[2] 14% moisture basis.
[3] The "as received" water retention values were corrected with the aid of the data in Table II to those values expected for Hobart flours having 1% more protein than the corresponding Buhler flours.

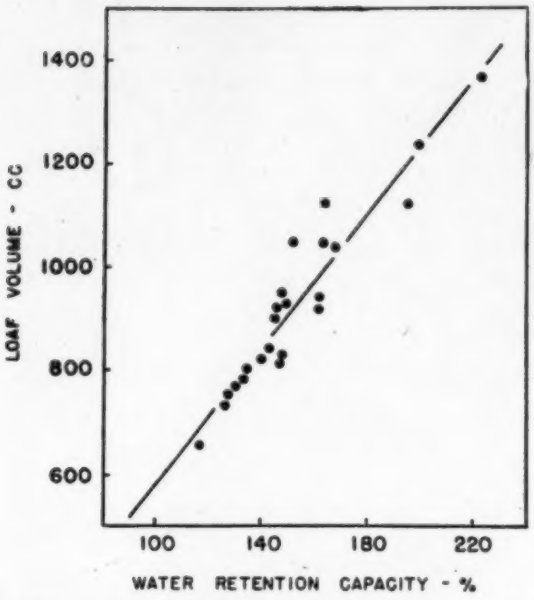

Fig. 4. Relation between Buhler flour loaf volume and Hobart flour water retention capacity for 23 hard red winter wheats representing two crop years and several protein levels for each of four varieties of varying protein quality (each water retention capacity has been corrected to a protein content 1% greater than that of the corresponding Buhler-milled flour by means of Table II).

In the practical application of the water retention capacity test, however, it must be considered in relation to the effect of variety and protein content. Accordingly, the "as received" water retention values and protein contents for the Hobart flours are plotted in Figure 5 for each of the four varieties (Table I). Pertinent statistical data are given in Table II. These data indicate that water retention capacity is a linear function of protein content within a variety.

For convenience in making comparisons, the regression lines of Figure 5 are repeated in Figure 6. These regression lines and their slopes (Table II) suggest a family of lines which tend to fan out from a common origin such that at a given protein content the slopes for the

TABLE II

CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS FOR WATER RETENTION CAPACITY AND PROTEIN CONTENT OF THE HOBART FLOURS REPRESENTING FOUR HARD WINTER WHEAT VARIETIES (x = PROTEIN CONTENT)

Variety	Correlation coefficient r	Regression equation
Comanche	0.91	$10.30x + 16.55$
Tenmarq	0.91	$8.02x + 42.78$
Cheyenne	0.96	$8.36x + 36.00$
Chiefkan	0.93	$6.56x + 43.30$

variety regression lines are positively correlated with water retention level (protein quality). These slopes are not different statistically, but a similar relation appears to exist for loaf volume and flour protein content (Finney, 1943) and has been established statistically for baking absorption and flour protein content (Finney, 1945). Thus, it appears justifiable to use the regression lines of Figure 6 as a basis for a correction chart representing all probable water retention capacity (protein quality) levels. Such a chart can be constructed by drawing in regression lines between and beyond those for Comanche and Chiefkan. By correcting the lactic acid water retention capacities of the Hobart

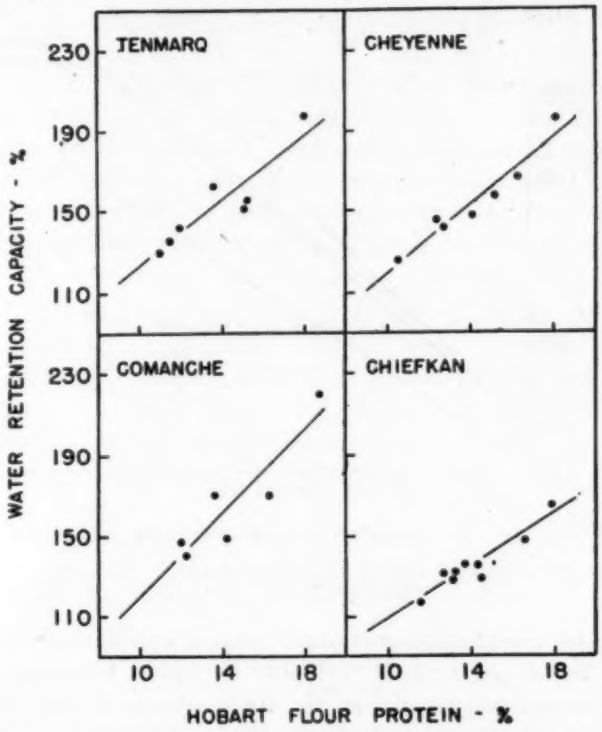

Fig. 5. Relation between water retention capacity and protein content of flours milled on a Hobart grinder from 29 hard red winter wheats representing four varieties of varying protein quality.

flours obtained from new wheat varieties to a constant protein basis such as 14½% (average for Hobart flours), an estimation of their protein quality or bread-making capacity relative to that for known varieties can be made quickly. As an example of the application of Figure 6 in making corrections for protein in variety evaluation, consider the data given in Table I for the last Cheyenne sample from Manhattan as representing a variety of unknown protein quality. Its "as received" water retention value of 142.1 at a protein content of

12.6% falls approximately on the Cheyenne regression line, thereby classifying the flour as being equal to Cheyenne in protein quality. The value of 142.1% retention is increased to 157% by following up the Cheyenne regression line to 14½% protein, at which point the water retention values of known and experimental varieties might be compared with a minimum of correction when considering all samples.

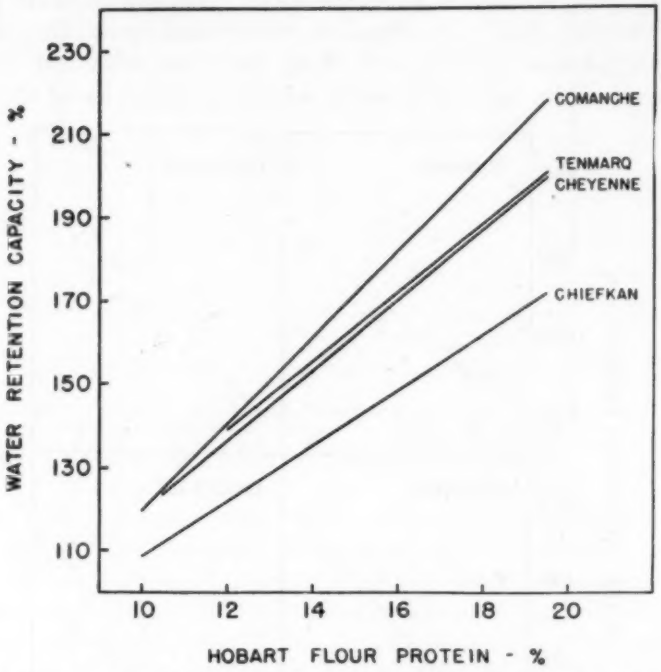

Fig. 6. Water retention capacity–protein content regression lines calculated from the Hobart flour data plotted in Figure 5.

The water retention capacity test appears to have excellent possibilities of being particularly valuable in wheat breeding where the limited amounts of material in the early phases of the program are insufficient for the usual milling and baking procedures. The test on Hobart flours also has possibilities in the inspection and purchasing of wheat in territories where undesirable varieties are grown.

Summary

Water retention capacity and loaf volume data are reported for samples of hard winter wheat varieties covering a wide range in protein content derived from a very wide range of climatic and soil conditions, and representing three crop years.

Water remaining imbibed after the application of centrifugal force is determined by treating 5 g of flour in a test tube with water to which

lactic acid is later added. After centrifuging and pouring off the supernatant liquid, water retention capacity is determined from the increase in weight.

Water retention capacity of Buhler experimentally milled flours is a reliable index of their loaf volume potentialities. Partial and multiple correlations indicate that water retention is an expression of both the quantity and quality of protein. The water retention values range from 150% for the flour producing the lowest loaf volume of 650 cc to 300% for the one giving the highest volume of 1365 cc.

A milling procedure involving one break and one reduction on the Hobart grinder and requiring only 11 to 12 minutes is described for 75 g wheat samples.

Water retention capacity of Hobart-milled flours is essentially a linear function of protein content within a variety. However, certain varieties have distinctly different regression lines, the slopes of which, in general, increase as water retention capacity increases.

Loaf volume of Buhler- and water retention capacity of Hobart-milled flours are very highly correlated when protein content is held constant. Accordingly, the differences between the water retention capacity regression lines apparently represent differences in protein quality.

These water retention capacity–protein content regression lines of known varieties should be useful for estimating the protein quality or breadmaking capacity of new wheat varieties.

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COMPOSITION OF HYBRID CORN TASSELS

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In the production of hybrid seed corn it is customary to plant alternately two rows to serve as the male parent and six rows to serve as the female parent plants. Tassels are removed from all female functioning plants as soon as they emerge or before pollen begins to shed, and thus before the subsequent fertilization is effected by the male functioning plants. Detasseling is carried out manually, the stalk generally being broken at the first node, and the tassels are discarded. Although the tassel comprises a relatively small portion of the total plant weight, the acreage devoted to seed production, and thus the quantity of tassels produced annually, is appreciable. Tassels of the particular strain investigated had an average dry weight of 0.033 pound per plant and since there are some 11,000 plants per acre, 75% of which are detasseled, the yield of dry tassels per acre was about 270 pounds. In 1944 there was an estimated 360,000 acres planted in hybrid seed corn which would have supplied nearly 50,000 tons of dry tassels. The tassels thus harvested can be retained at little extra cost and dried if necessary in seed corn driers which are idle during the detasseling period. It appeared desirable, therefore, to investigate their composition with a view toward utilization as a feed adjunct. Analyses were carried out during three stages of tassel development, the intermediate stage being the normal detasseling time. Also analyzed for comparative purposes were corn pollen and commercial hybrid corn grain. Tassels were found to be a good source of protein and vitamins and their possible utilization as a feed component is discussed.

Experimental Methods

About 20 tassels were taken for each analysis. Rachises below the lowest spikelet were removed so as to provide a more uniform material for analysis. It should be noted, therefore, that the analytical data presented later refer only to the tassel, rather than to the tassel, lower portion of rachis, and leaf, which are generally removed in detasseling.

Nitrogen, crude fat, and crude fiber were determined by the *Methods of Analysis* (Association of Official Agricultural Chemists, 1940). Ash was determined by holding at 550°C for four hours and moisture

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by drying overnight at 105°C. Carotene was measured by the method of Moore (1940) and thiamine by the fermentation method of Schultz, Atkin, and Frey (1942). The method of Snell and Strong (1939) was employed for the riboflavin assay after the samples were extracted by autoclaving at a pressure of 15 pounds for 30 minutes in the presence of 0.1 *N* hydrochloric acid. Pantothenic acid was determined by the method of Pennington, Snell, and Williams (1940) after digestion of the samples with clarase and papain for 24 hours at 37°C. Results were corrected for the vitamin contained in the enzymes. For niacin, the procedure of Snell and Wright (1941) was employed. Samples were previously hydrolyzed by autoclaving for 30 minutes at 15 pounds pressure in 0.1 *N* sodium hydroxide. For the pyridoxine assays, samples were hydrolyzed by autoclaving with 5.0 *N* sulfuric acid after which the method of Atkin, Schultz, Williams, and Frey (1943) was applied.

Results

The results of proximate analyses of tassels during three stages in development are presented in Table I. Also included for comparison in Table I is the analysis of a composite sample of corn grain made up of over 100 commercial and experimental hybrids.

TABLE I

INFLUENCE OF MATURATION UPON THE COMPOSITION OF COMMERCIAL HYBRID CORN TASSELS
(DeKalb Strain 404A)

Stage of tassel development	Proximate analysis					
	Moisture [1]	Protein (*N* x 6.25)	Fat	Ash	Crude fiber	Nitrogen-free extract
	%	%	%	%	%	%
Early [2]	70.9	18.5	5.8	4.5	22.8	48.4
Intermediate [3]	55.9	18.3	6.9	4.5	18.2	52.1
Late [4]	54.6	17.1	4.3	4.6	18.5	55.5
Yellow corn, whole grain [5]	10.5	10.4	5.0	1.4	2.1	81.1

[1] Moisture is given on the "as received" basis; all other constituents on a dry basis.
[2] Tassels were entirely green and pollen was immature.
[3] Pollen mature but firmly held (normal detasseling stage).
[4] Pollen mature and released by handling tassel.
[5] Composite sample of hybrids.

The solids content of tassels increased substantially between the early and intermediate (detasseling stage) and thereafter remained relatively constant. While the highest solids content of tassels as harvested at the intermediate stage was only 44.1%, it was found that samples could be dried to more than 90% solids by exposure in the field. Except for their lower content of carotene, as will be shown later, sun-

dried samples were similar to oven-dried samples in all constituents. The protein content decreased slightly during maturation but in all instances was notably high compared with the grain or other structural parts of the corn plant. The fat level rose to 6.9% at the detasseling stage, then decreased rather sharply during further development of the tassel. The ash content remained practically at the same level throughout. Early tassels were higher in crude fiber than intermediate or late tassels, probably because there was a higher proportion of rachis tissue in the immature tassels. The nitrogen-free extract increased slightly during the period of development, and at the detasseling stage it constituted approximately one-half the total dry substance.

The vitamin content of tassels (Table II) also was found to be

TABLE II

INFLUENCE OF MATURATION UPON THE VITAMIN CONTENT OF COMMERCIAL HYBRID CORN TASSELS
(DeKalb Strain 404A)

Stage of tassel development [1]	Vitamin content [2]					
	Riboflavin	Niacin	Pantothenic acid	Pyridoxine	Thiamine	Carotene
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
Early	6.5	58.0	23.0	3.9	6.1	—
Intermediate	8.8	60.0	25.9	3.2	10.0	13.0
Late	9.4	62.5	22.8	3.0	8.8	—
Yellow corn, whole grain [3]	1.1	21.7	9.1	7.4	5.4 [4]	1.1 [5]

[1] Stages of tassel development are explained in Table I.
[2] All analyses are given on a dry basis.
[3] Composite sample of hybrids.
[4] From Schultz, Atkin, and Frey (1941).
[5] From Baumgarten, Bauernfeind, and Boruff (1944).

unusually high when compared with other structural parts of the plant and, with the exception of pyridoxine, was considerably greater than that of corn grain. For example, tassels at the intermediate stage of maturation contained approximately eight times the riboflavin, three times the niacin, three times the pantothenic acid, twelve times the carotene, and twice the thiamine potency of the sample of corn grain. The pyridoxine level, however, was only about one-half that of the grain. While riboflavin and thiamine appeared to increase significantly during tassel development, the values for niacin, pantothenic acid, and pyridoxine remained relatively constant. The vitamin content of tassels which had been oven-dried was compared with those dried to 8% moisture content by exposure to the sun. Whereas no differences were noted in the B-vitamin content, the carotene level of sun-dried tassels was only 4 μg per gram or less than one-third that contained in the oven-dried material.

A question arises as to the origin and location of the vitamins in the tassel since this organ is comprised of several parts which differ in physiological function such as the rachis branches, glumes, anthers, pollen, etc. Although these fractions were not separated and analyzed, the potency of pure corn pollen in several members of the B complex was determined and found to be as follows per gram of dry substance: 14.9 μg riboflavin, 81 μg niacin, 30 μg pantothenic acid, and 4.7 μg pyridoxine. Except for pyridoxine which is lower in corn pollen, these figures are in good agreement with those reported for mixed pollen by Kitzes, Schuette, and Elvehjem (1943). The vitamin content of pollen is but little greater than that of the whole tassel and does not entirely explain the high potency of the blended parts.

While the analytical data indicate that dried tassels are superior to corn and other cereal grains which are more readily available feed constituents, they are not comparable to such feed concentrates as distillers' solubles or dried skim milk in either their protein or water-soluble vitamin content. Therefore, the value of this material probably will be less than these adjuncts but will be determinable only by actual feeding trials. The potency of dried tassels in members of the B-vitamin complex suggests that they may be suitable for supplementing poultry rations.

Summary

The composition of hybrid corn tassels at various stages of maturation has been determined. Tassels are good sources of protein and vitamins, and they attain highest nutrient value at the normal detasseling time. Dried tassels may be a suitable adjunct to poultry feeds.

Acknowledgment

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GAS PRODUCTION AND GAS RETENTION OF DOUGH AS AFFECTED BY TYPE OF FLOUR, BAKING FORMULA, AND AMOUNT OF MIXING[1]

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Gas production and gas retention are basic to the production of leavened bread. Harden (1911) found that for a given amount of yeast the rate of fermentation is almost constant, regardless of the sugar concentration, within the limits of 0.5% to 10% sugar. Johnson and Bailey (1925) believed that the gluten content of flour directly affected the gas-retaining properties of dough but did not alter the total gas production. Heald (1932) listed factors that increased gas production as: (a) increased yeast concentration; (b) sugar and diastatic malts when a deficiency of each occurs; (c) yeast foods up to a certain amount; and (d) elevated temperatures up to 95°F (35°C). Those factors which decreased gas production were: (a) salt; (b) excessive amounts of yeast food; and (c) excessive temperature. Those factors which had no apparent effect were: (a) sugar and diastatic malt when sufficient amounts were already present; (b) high speed mixing; and (c) absorption between the limits which would be used in ordinary dough fermentation. Blish and Hughes (1932) reported that maintained gas production is predominantly dependent upon the supply of fermentable sugar. They further postulated that gas production is, for all practical purposes, independent of the gas retention factor under normal baking conditions. Munz and Bailey (1936) also refer to the effect of the addition of sugar on gas production and they agree with the findings of these other workers. Bohn and Bailey

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(1936) reported that overmixing produces marked physical changes in dough properties. The extent to which flour characteristics, baking formula, and procedure influence gas retention is not so well understood as the influence of these factors on gas production.

The purpose of the present investigation was to evaluate the influence of three factors on gas production and retention, namely: flour characteristics, formula, and mixing time.

Materials and Methods

The study was limited to a comparison of three commercially milled flours, three formulas, and three mixing times. The flours comprised a soft winter, a hard red spring, and a hard red winter, which were selected as representatives of each type from a group of some twenty available samples. Selection of the three flours was made to secure differences in protein content, diastatic activity, absorption, and mixing requirements. These data are recorded in Table I.

TABLE I

CHARACTERISTICS OF WHEAT FLOURS[1]

	Flour type		
	Soft winter	Spring	Hard winter
Protein, %	7.8	13.6	13.6
Ash, %	0.42	0.49	0.45
Moisture, %	11.6	11.6	11.7
Diastatic activity, mg/10 g	130	195	347
Optimum mixing time, min.	2.0[2]	2.5	3.5
Absorption (lean formula) %	54.5	72	64

[1] Data are expressed on the "as received" basis.
[2] Two minutes was arbitrarily taken as the optimum mixing time for the soft wheat flour. Actual measurements indicated a shorter time. However, the apparatus was geared to testing hard wheat flours and it was difficult to obtain a properly mixed soft wheat dough that would be differentiated from the one-minute mixing given to "undermixed" doughs. A mixing time of less than one minute was not practical because of insufficient incorporation of the ingredients and poor handling properties.

One low-sugar and two high-sugar formulas, designated respectively as "lean," "MPB," and "rich" with the ingredients shown in Table II, were used. With both high-sugar formulas the initial rapid rate of gas production was maintained for at least eight hours. The only instances in which sugar deficiencies occurred were with the "lean" formula.

The optimum mixing times were estimated from the characteristics of mixograms and by the handling properties of the dough as judged by an experienced baker. A dough was judged to be at optimum development when it had a smooth texture and dull surface, and felt dry when handled. In addition to doughs mixed for the optimum times for each flour and formula, undermixed doughs (mixed one minute) and overmixed doughs (mixed 10 minutes) were also prepared.

TABLE II

BAKING INGREDIENTS PER 100 GRAMS FLOUR

	Lean	MPB	Rich
	g	g	g
Yeast	2.5	2.5	2.5
Sugar	—	5.0	6.0
Salt	—	1.0	1.5
Dry milk solids	—	—	6.0
Malt syrup			
120°L	—	—	0.25
200°L	—	0.30	—
Ammonium phosphate	—	0.10	—
Bromate	—	1 mg	4 mg
Water		As needed	

One 5-g aliquot of each dough was used to estimate gas production, and another to estimate gas retention employing the recording gasometer recently described by Working and Swanson (1946). The data thus obtained were calculated to a 170-g dough basis, this being the average weight of the dough from 100 g of flour. Gas production data for the flours are reported as: (1) rate per hour for the initial phase, that is, up to the point where the curve breaks and the rate decreases; (2) six-hour total production; and (3) total production to the point of maximum gas retention. The latter may have considerable significance since it is dependent not only on the gas-producing ability of the dough but likewise on the gas-retaining capacity. The gas retention values recorded were the maximum quantity of gas retained and the time necessary to reach this maximum.

Results

The mean data for gas production and gas retention are given in Table III. Each numerical value represents the combined mean data from 54 determinations (three flours tested in duplicate with three formulas and three different mixing times).

Gas Production. The soft wheat flour showed the lowest initial rate of gas production and a low total production, both to the point of maximum retention and to six hours. The other two flours had very similar initial rates of production, but the high diastatic winter wheat flour maintained the initial rate of production for a longer time and, thus, gave the most total gas produced during the six-hour period. The value for total production to maximum retention was greater for the spring than for the winter wheat flour owing to the longer time required to attain maximum retention.

The type of formula had a pronounced effect on the initial rate of fermentation and on the total gas produced. The initial rates for the lean and MPB formulas were essentially the same, but the rate for the

TABLE III

EFFECT OF FLOUR TYPE, BAKING FORMULA, AND MIXING TIME ON GAS PRODUCTION AND RETENTION
(mean results for 170 g dough)

Main effects	Gas production			Gas retention	
	Initial rate	Total to		Maximum	Time to maximum
		Maximum retention	Six hours		
	ml/hr	*ml*	*ml*	*ml*	*hr*
Flour					
Soft	227	692	1149	298	3.1
Spring	239	1060	1273	451	4.6
Winter	241	961	1314	371	4.0
Formula					
Lean	250	728	1002	319	3.0
MPB	251	1048	1277	427	4.2
Rich	209	939	1457	375	4.6
Mixing time					
1 minute	234	806	1226	322	3.6
Optimum	236	1019	1253	416	4.3
10 minutes	239	889	1257	384	3.8

rich formula was much lower. The gas production values to the time of maximum retention and for six hours were highest for the MPB formula and lowest for the lean formula. This was due to the influence of the lower rate of gas production with the rich formula and to the shorter time to maximum retention with the lean formula.

Variations in dough mixing time did not influence the initial rate of gas production nor, to any appreciable extent, the six-hour production values. However, the total gas production to the point of maximum retention was largest for the optimum mixing time, smallest for the one-minute, and intermediate for the 10-minute mixing time. This is due to the effect of the mixing on the time elapsing between the beginning of fermentation and the attainment of peak retention; this time was the longest with optimum mixing and the shortest with undermixing.

Gas production, then, over either the variable time required to give peak retention or during the conventional six-hour period, is dependent primarily on the formula used. The type of flour may have an effect but this can be modified by changing the formula.

Gas Retention. Of the three flours, the spring wheat sample showed the greatest gas retention, the winter wheat flour intermediate, and the soft wheat flour the lowest. This relationship, in part, results from the dependence of gas retention, as measured, on both gas production and the time required to reach the retention peak. The soft

wheat flour was deficient in both respects; the spring wheat flour was not superior to the winter in early gas production rate, but the longer period of time before the retention peak was reached permitted greater gas production and greater observed retention.

The maximum gas retention was largest for the MPB formula, smallest for the lean, and intermediate for the rich. Here again, the variation may be attributed primarily to differences in gas production. The effect of the lean formula was to give a low over-all gas production during the relatively short period before maximum gas retention was achieved. High gas production for a relatively long period of time caused the observed retention peak for the MPB formula to be high. On the other hand, with the rich formula the prolonged fermentation period before peak retention was reached was not sufficient to overcome the deficiency caused by the lower rate of gas production; as a result the retention for this formula was of intermediate degree.

Best gas retention was observed with the doughs mixed for the optimum, while the undermixed doughs showed lowest retention and the overmixed gave values of intermediate magnitude. Variation in mixing time had, essentially, no effect either on the rate of gas production or on the period of maintenance of this rate. Observed differences in maximum gas retention must therefore be due to an influence on the gas-retaining properties of the dough. Thus, gas retention is shown to be dependent primarily on gas production and mixing time, and secondarily on the flour.

Variance Analyses

Results of variance analyses of the data are summarized in Table IV. The interactions were tested by the "remainder" error term.

The variance analyses confirm the observations made from the data of Table III and only the significant interrelationships of flour, formula, and mixing time will be discussed. Figure 1 illustrates these relationships for total gas production up to the time of maximum retention. The flour-formula graph (left side) shows the superior response of the spring wheat flour to the MPB and rich formulas, the low response of the winter wheat flour, and the intermediate response of the soft wheat flour. The flour-mixing time graph (center) shows the superiority of optimum mixing time for the winter and spring wheat flours. For the soft wheat flour, there was no difference in response to the one minute and optimum mixing times, while the 10-minute mixing time was clearly damaging. The spring wheat flour was most sensitive to changes in mixing time. The formula-mixing time graph (right side) shows no effect of mixing time with the lean formula, but with the rich and MPB the optimum mixing time is clearly shown to be superior to under- or overmixing.

TABLE IV

VARIANCE ANALYSIS OF GAS PRODUCTION AND RETENTION DATA

Source of variation	D.F.	Sums of squares [1]						
		Gas production			Gas retention		Duration of initial rate of production (data from lean formula only)	
		Initial rate	Total to maximum retention	Total to six hours	Maximum	Time to maximum	D.F.	
Flours	2	2.75*	1973	846	317	34.3	2	10.92**
Formulas	2	26.57**	1436*	6536*	159	38.3*	—	—
Mixing times	2	.24	621	31	123	7.4	2	.01
Interactions								
Flour × formula	4	.52	190*	566**	17*	4.3**	—	—
Flour × mixing time	4	.80	471**	23	65**	6.2**	4	.06
Formula × mixing time	4	.17	199*	22	28**	2.4*	—	—
Remainder	143	.44	27	23	6	.5	45	.06

[1] **Denotes that variances are highly significant (exceed 1% point).
*Denotes that variances are significant (exceed 5% point).
The interactions were tested for significance against the "remainder" error term. The main effects were tested for significance by the appropriate interactions.

Figures 2 and 3 illustrate the relationships for maximum gas retention and time to maximum retention, respectively. The flour-formula graph (left) of Figure 2 shows the soft wheat flour to have the lowest gas retention for all three formulas with the spring wheat flour superior to the winter. The significance of the interaction is due to the difference in *relative* sensitivities of the flours to formula changes. The flour-mixing time graph (center) shows the greatest retention with the winter and spring wheat flours at the optimum mixing time, the least at the one minute, and intermediate retention at the 10-minute mixing time. The soft wheat flour has the least retention, the spring the most, with winter intermediate. The mixing time-formula

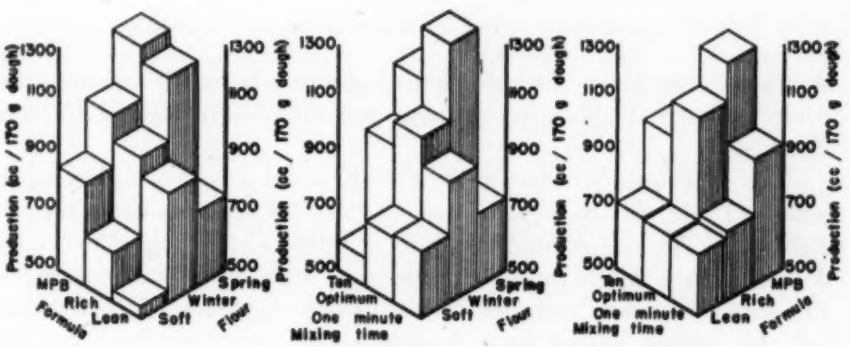

Fig. 1. Flour, formula, and mixing time relationships for total gas production up to the time of maximum retention.

graph (right) shows about the same relationship as the flour-mixing time graph (center).

The left graph of Figure 3 (time to maximum retention) shows the high sensitivity of the hard spring and soft winter wheat flours to formula changes, with the winter wheat flour least affected. The significance of the flour-mixing time interaction (center graph) was due, mainly, to the insensitivity of the flours at one-minute mixing as compared to their rather wide differentiation at either optimum or ten-minute mixing. The formula-mixing time interaction (right graph)

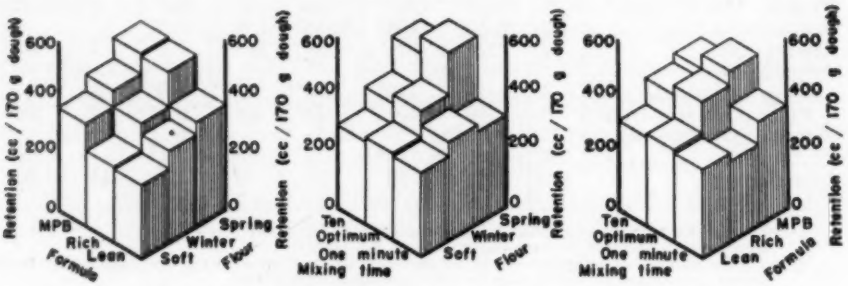

Fig. 2. Flour, formula, and mixing time relationships for maximum gas retention.

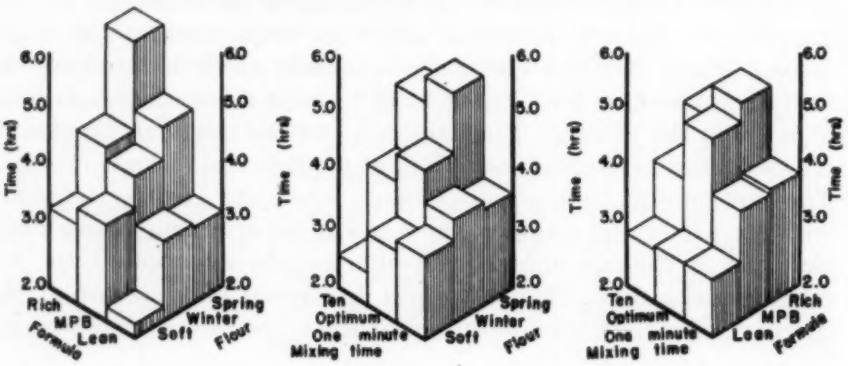

Fig. 3. Flour, formula, and mixing time relationships for the time to maximum gas retention.

shows the lean formula to be unaffected by variations in mixing time, while both the high-sugar formulas were markedly altered by such changes.

The flour-formula interaction for the total gas production to six hours (Table IV) was highly significant. The data are given in Table V. The lean formula gave the smallest values regardless of the flours, and the MPB formula the largest, with the rich intermediate. The soft wheat flour gave the lowest values regardless of the formula, but the highest values were determined by both the flour and the formula, with the winter wheat flour having the greatest value with

TABLE V

EFFECT OF FLOUR CHARACTERISTICS AND BAKING FORMULA ON TOTAL GAS PRODUCTION

Formula	Gas produced in six hours		
	Flour		
	Soft	Winter	Spring
	ml	*ml*	*ml*
Lean	848	1220	929
MPB	1392	1460	1537
Rich	1220	1258	1370

the lean formula and the spring wheat flour the greatest with both the MPB and rich formulas.

The data show the extent to which gas retention is influenced by the rate of gas production and the duration of the initial rate. The correlation of gas production and gas retention was highest at the time of maximum retention, being + 0.83. The correlation at six hours was + 0.30. This suggests that gas production data, when used as a criterion of potential breadmaking quality, should be noted at some time less than the usual practice of six hours.

Summary

Gas production and retention data were obtained for three wheat flours varying in protein content, mixing requirement, and diastatic activity employing a "lean" (low-sugar), malt-phosphate bromate, and a rich formula and short, optimum, and long mixing times.

Gas production was dependent, primarily, on the formula used. The rich formula had the lowest rate of gas production, while the lack of fermentable sugar with the lean formula caused a marked decline in the amount of gas produced. The effect of flour type on gas production can be modified by changing the formula.

Gas retention was primarily influenced by gas production and mixing time. The higher the rate of gas production the greater the *amount* of gas retained. Optimum mixing produced a dough which had the best gas-retaining properties.

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REPORT OF THE 1945-46 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

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(Presented at the Annual Meeting, May 1946; received for publication May 23, 1946)

Several years have been spent in an attempt to reconcile laboratory and shop performances in cooky baking. Some correlation has been evident with our present tentative cooky formula, particularly with wire-cut goods (Schwain, 1944), but this formula has revealed a sensitivity to flour particle size not always demonstrated in the bakery. Last year, limited studies with a typical shop formula reduced to laboratory size showed better correlation with actual practice in this regard (Schwain, 1945).

Any attempt to explore the question of flour particle size this year had to be abandoned. Actual mill runs by Wilbur Hanson, one of the committeemen, demonstrated that not over 10 to 15% variation in flour particle size could be obtained from a given lot of this year's crop of Michigan white wheat.

The committee then turned to a problem which has confronted the industry for some time, namely, the tolerance range of cooky flour strength. In other words, how far apart in analyses can cooky flours be and yet produce cookies of equivalent spreading properties? It was decided to select the test flours on the basis of gluten quality or strength as defined by apparent viscosity, and to determine the actual tolerance range by cooky tests in both the laboratory and shop. A significant change in spreading properties of the cookies from any given flour was to be considered as an indication that its strength or weakness, as the case might be, was of sufficient magnitude to place it outside the range. Conversely if no appreciable change in baking response was noted, one could assume that the strength of the flour in question fell within the range of tolerance. Wire-cut types of cookies

were chosen as the medium for the shops to explore in view of the previous good correlation between laboratory and bakery results with this type of piece.

Materials and Methods

Four unbleached straight-grade Michigan white wheat flours were selected which had apparent viscosities by the no-time method ranging from 22° to 34° MacMichael.

The analyses of the test flours are shown in Table I and a Ro-Tap granulation study in Table II. The "no-time" viscosity test ranks flour *A* the weakest and *D* the strongest, with *B* and *C* about equal. The "one-hour" viscosity, on the other hand, ranks *A* and *B* about on a par, with *C* stronger than these two but *D* again showing the greatest strength. It is possible that the higher mineral content of flour *A* is reflected in a lowering of its no-time viscosity. In any event a considerable range in apparent viscosity or gluten strength is indicated.

TABLE I

ANALYSES OF TEST FLOURS

Flours	Ash [1]	Protein [1]	Gluten		Maltose value [2]	Apparent viscosity	
			Wet	Dry		No-time	1 Hour
						°MacM.	°MacM.
	%	%	%	%	*mm Hg.*		
A	0.42	7.3	23.6	7.9	275	22	38
B	0.38	7.0	23.2	7.8	305	25	37
C	0.36	6.0	21.3	6.7	340	25	44
D	0.36	7.1	22.9	8.2	340	34	51

[1] 14% moisture basis.
[2] As determined by pressuremeter in 6 hour period.

TABLE II

GRANULATION OF TEST FLOURS [1]

	Percent of total flour			
	Flours			
	A	*B*	*C*	*D*
Over 11 XX Silk	0.3	0.5	3.3	0.4
Over 12 XX Silk	0.6	0.8	3.1	1.1
Over 13 XX Silk	1.4	3.7	9.4	3.9
Over 14 XX Silk	0.4	3.4	1.5	4.8
Over 15 XX Silk	7.7	14.4	15.8	15.5
Through 15 XX Silk	89.6	77.2	67.0	74.3

[1] Flours were bolted for 30 minutes by means of a Ro-Tap shaker (200 g sample).

Granulation differences doubtless reflect variations in grinding and bolting operations since three different flour mills are represented.

Mixograms made from the four flours are reproduced in Figure 1. These were all made at an absorption of 52% and at a machine tension of 7. The slower development time of flour *C* is probably the result of its coarser granulation.

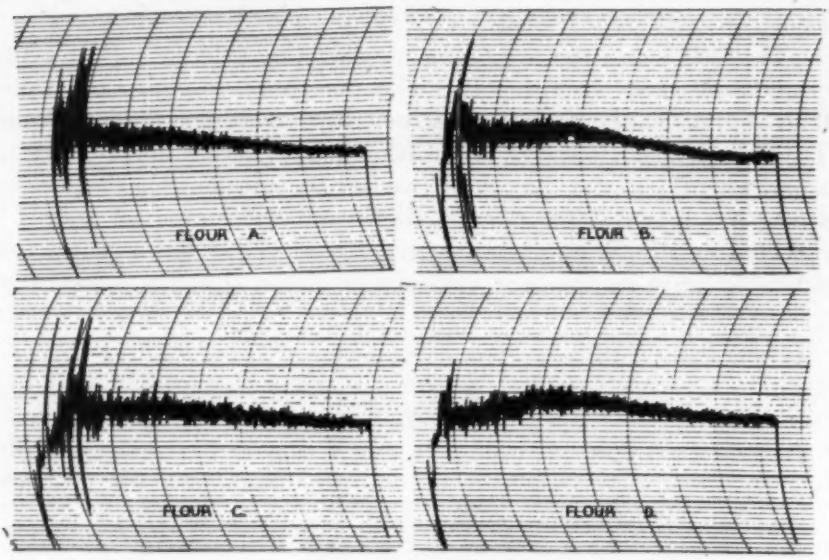

Fig. 1. Mixograms of test flours (tension 7; 52% absorption).

Cooky tests were conducted in five different laboratories and five separate shops.

The laboratory cooky baking tests were carried out in three ways. The first method consisted of the present tentative test first proposed by Hanson (1943). To this was added the modification employed by last year's committee to secure a more uniform dough consistency by controlling the amount of dry matter and the quantity of moisture added (Schwain, 1945).

The second method was fundamentally a shop wire-cut piece formula reduced to laboratory size. This is the same formula, essentially, that the committee had tried last year, except that dough consistency was controlled in the same way as in the case of the formula mentioned above. The primary difference between the present tentative laboratory formula and the shop revision was in the amount of leavening agents. The shop type utilized a far greater amount of ammonium bicarbonate and a lesser quantity of sodium bicarbonate. These changes encourage more spread than spring in the resulting cookies.

A third laboratory method consisted of a micro-cooky test developed by Karl Finney at Wooster. In this method, the dough from any given formula is mixed in a National nonrecording mixer with only two cookies baked from the same dough.

Since unpublished work has shown that the kind of metal used for cooky baking sheets produces a noticeable effect on the spring and spread of cookies, the committee decided to use a 20 gauge iron sheet in its laboratory tests. This type of metal is the same as that normally employed in baking pans for use in commercial reel type ovens.

The various bakeries were asked to employ their regular wire-cut formulas but to make no changes except possibly in absorption or, if necessary, in the setting of the wire-cut machine feeder rolls to secure satisfactory results.

Results

The results from the laboratories, recorded in Table III, show that the flours are remarkably close in cooky baking performance. Both formulas show reasonably good agreement. The revised shop formula, as was expected from its leavening balance, produced the greater

TABLE III

LABORATORY SPREAD FACTORS (W/T) OF COOKIES MADE WITH THE PRESENT TENTATIVE TEST AND THE REVISED SHOP FORMULA

Collaborator	Spread factor of cookies							
	Flours							
	A		*B*		*C*		*D*	
	Tent.	Revd.	Tent.	Revd.	Tent.	Revd.	Tent.	Revd.
I	11.2	10.9	11.3	10.7	10.7	11.5	11.2	11.7
II	9.4	9.7	9.4	10.2	9.0	10.3	9.4	10.1
III	9.0	10.2	8.7	9.8	9.3	10.2	8.4	10.0
IV	8.9	9.6	9.1	9.7	10.0	9.6	10.4	9.9
V[1]	9.4	9.6	8.8	9.5	8.8	9.4	8.9	9.2
General average	9.6	10.0	9.5	10.0	9.6	10.2	9.7	10.2

[1] Micro cooky baking test technique employing same formulas.

spread. The micro-test shows better agreement with the revised shop formula averages than with the present tentative test. Individual results in general are not in as close agreement as in prior years.

Actual shop tests of the flours are summarized in Table IV.

Admittedly the choice of cookies by the various shops is not representative of the best types that could have been chosen; but the bakeries are still under considerable handicaps as far as basic ingredi-

TABLE IV

SPREAD FACTORS (W/T) OF WIRE-CUT COOKIES FROM COMMERCIAL TESTS

Collaborator	Type cooky	Flours			
		A	*B*	*C*	*D*
VI	Vanilla wafer	5.8	6.6	6.0	5.5
VII	Vanilla wafer	6.7	6.9	6.7	6.7
VIII	Oatmeal	7.6	7.7	7.6	7.6
IX	Oatmeal	8.0	8.5	—	8.0
X	Coconut	8.1	9.4	9.2	8.7
General average		7.2	7.8	7.4	7.3
Flour moisture		11.3	13.5	12.8	12.6

ents are concerned. Oatmeal doughs sometimes are not as sensitive to flour types as most other formulas. However, the average values for the shop tests show that with the exception of flour *B*, the samples are very similar in cooky making value.

It is noteworthy that flour *B* had the highest moisture and it is quite likely that this is a contributing factor to the high spread since all of the bakeries employed the flours on an "as received" basis. All the bakeries reported that the dough from flour *B* was on the soft side. Two percent additional moisture is equivalent to using 12 pounds less flour and 12 pounds more water per three barrel dough and this would naturally lead to a softer dough with more spread.

This suggests that it would be advantageous for bake shops to weigh flour into the mixers with a weight standardized to some predetermined moisture level, say 12%. Such a procedure might conceivably level out flour performance; it would certainly aid in the attainment of more uniform goods, and should reduce invisible losses which ultimately affect yield.

Collaborator X had sufficient flour to repeat the flour series, in which he prepared each dough by employing a constant weight of flour as computed on a 12% moisture basis. The results are found in Table V.

TABLE V

COMPARISON OF W/T FACTORS FOR WIRE-CUT COOKIES FROM COMMERCIAL DOUGHS CORRECTED AND UNCORRECTED FOR FLOUR MOISTURES

Dough preparation	Flours			
	A	*B*	*C*	*D*
Flour weighed "as received"	8.1	9.4	9.2	8.7
Flour weight corrected to 12% moisture basis	9.6[1]	9.5	9.7	9.5[1]

[1] Average of several doughs.

When moisture is taken into account in the shop, the performance of the different flours is much more uniform. This is in harmony with the laboratory predictions. Flours *A* and *D* were baked in duplicate in the shop and the replicability of the tests on flour *A* was very unsatisfactory; W/T values of 10.15 and 9.0 were secured in successive days under strict supervision. This points clearly to the fact that the shop data, which in most cases represents single doughs, may not reflect what would be found as a result of a large number of tests where such unavoidable variations as personnel, machine, and baking conditions would tend to be overcome.

Conclusions and Recommendations

From these studies it would appear that unbleached Michigan white wheat straight grade flours may vary in apparent viscosity as much as 10° MacMichael on the basis of the no-time method or approximately 15° MacMichael with the one-hour digestion test and still produce wire-cut cookies with comparable spreading properties. This has been demonstrated in laboratory tests and somewhat limited commercial runs. The latter showed quite clearly that the performance of flours may be markedly affected by differences in moisture content, particularly if the moisture variable is overlooked by the bakery in weighing the flours for the doughs.

Since moisture is usually neglected in the shop, it is well for the mill chemist to keep this variable in mind should a criticism be leveled against his flour when compared to another similar type. Conversely, the cooky bakeries would do well to consider the moisture factor when weighing the flour since it is obvious that to ignore it is to invite variations in performance, quality, and actual yield.

Both laboratory formulas agree fairly well in evaluating the flours. The results support the view held by many soft wheat workers that no single physical or chemical test will define the cooky performance of flours, and that the baking test is one of the most dependable guides.

It is recommended that future committees continue to work toward an official cooky test bake formula, studying both formulas used here, with the object of eventually recommending the one which is most suitable. The ammonium bicarbonate tolerance of a flour might be investigated. The range of cooky flour strength employing flours with differences in viscosity of about 15° MacMichael (no-time), if such a spread can be obtained from Michigan white wheat, should be further explored. Closer agreement in laboratory results was secured in prior years when only two or three flours were studied than in the present study which involved a heavier schedule. Shop test accuracy would also be improved by making replicate tests with fewer flours.

Acknowledgments

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BOOK REVIEW

Studies on the Nature of the Bromate Effect. By Holger Jørgensen. 435 pp. English Edition published in 1945 by Einar Munksgaard, Copenhagen, Denmark, and Humprey Milford, Oxford University Press, London, England. Price "Dan. Kr. 40."

In this voluminous text Holger Jørgensen reviews and amplifies his previous work on the mechanism of the action of bromate and other improvers on flour. The English edition published in 1945 is a translation from the Danish by Einar Christensen of Jørgensen's doctoral thesis published in Copenhagen in 1941.

The book consists of six introductory chapters, three main sections, an appendix describing the flour inventory, and finally a bibliography. The first main section is concerned with the methods used in the investigation. Baking test procedures and methods for the determination of nitrogen, moisture, ash, diastatic activity, hydrogen ion concentration, and oxidation-reduction potential are discussed in minute detail. Techniques employed for the measurement of the activity of papain and similar proteolytic enzymes by means of both gelation and extraction tests are fully outlined. In the second section the author cites numerous experiments supporting his contention that the beneficial effect of bromate and other improvers is due to inhibition of the flour proteinases which have been activated by the presence of yeast. The arguments he gives in support of this theory are as follows:

"(1) It can be established indisputably by tests that the action of the oxidizing agents which improve the baking strength is *not* due to the circumstance that these substances stimulate the CO_2 production of the yeast. Thus we may reject as erroneous one of the oldest theories for the bromate effect, viz., that bromate incites the yeast to a stronger CO_2 production.

(2) It can be established by test that $KBrO_3$, KIO_3, $(NH_4)_2S_2O_8$ and $NaBO_3$, $4H_2O$ (thus all of the oxidizing improvers) strongly inhibit the gelatin-splitting ability of papain and its related enzyme, bromelin. $KClO_3$, which does not improve the baking strength, does not inhibit the action of these proteinases.

(3) When extracting flour with water (autolysis) the improvers $KBrO_3$ and KIO_3 cause a diminished solubility of the N of the flour, while the not-improving oxidizing agent $KClO_3$ does not have this effect on the N-solubility.

(4) The depression of the N-solubility due to the addition of $KBrO_3$ and KIO_3 mentioned under (3) increases very considerably if the extraction is carried out on wheat flour which has been made strongly proteolytic by the addition of papain. $KClO_3$, however, remains passive.

(5) It can be established by test that the proteolytic influence of wheat germ and wheat malt on gelatin is inhibited by $KBrO_3$ and KIO_3, but *not* by $KClO_3$.
(6) Ascorbic acid—a substance of which it is known that it can inhibit proteinases of the papain group—has been found to act improving on the baking strength.
(7) Glutathione—a substance of which it is known that it activates proteinases of the papain group—has been found to destroy the baking strength.
(8) By its presence in dough, yeast causes an activation of the proteinases of the flour."

The third main section is a critical review of 23 papers by other investigators dealing with Jørgensen's theory.

It has been demonstrated by numerous investigators that proteolytic enzymes are present in wheat and, as might be expected, they occur in a smaller amount in patent flour than in other milled fractions of the wheat. Moreover, all evidence points strongly to the fact that the proteolytic enzymes of wheat belong to the papain group. Previous workers have shown that enzymes classified in the papain group are activated by sulfhydryl compounds and cyanide and inactivated by certain oxidizing agents or other compounds containing elements such as copper or mercury that can combine with the sulfhydryl group of the enzyme. But the fact that certain oxidizing and reducing agents act solely by inhibiting or activating the proteolytic enzymes of flour is difficult to prove, because every method of measuring the change in the proteolytic activity of flour, as applied to the complex conditions existing in breadmaking, is open to some criticism. The proteolytic activity of patent flour is small and differences with various flour treatments are not significant unless levels many times those used in commercial practice are employed. Jørgensen has attempted to answer this criticism by pointing out that the ratio of water to flour is much greater in his extraction experiments than in dough and that the presence of yeast in dough would further activate the enzymes and hence less oxidizing agent will be required. However, it appears to the reviewer that both these arguments prove the opposite. In solution there should be a greater chance for a more complete reaction between the enzyme and the oxidizing agent than in a dough. Since yeast activates the proteolytic enzymes of flour, one would think that greater amounts of oxidizing agents would be required in dough than in the extraction tests; yet the opposite is the case. Secondly, gluten is a most difficult substrate to use for the measurement of proteolytic activity. Most workers in this field have had to resort to the use of foreign substrates such as casein or gelatin. Jørgensen has leaned heavily on two techniques for demonstrating his thesis. One is an extraction procedure in which flour is autodigested in aqueous suspension with and without the improver and the difference in the amount of soluble nitrogen in aliquots of the centrifuged, filtered extracts is determined. The other method is a procedure whereby proteolytic activity is measured qualitatively by the ability of the flour suspension, with or without additions, to change the time of solidification of gelatin.

Jørgensen's weakest arguments are those listed under (2), (4), and (7). The inhibition of the gelatin-splitting ability of papain and bromelin by bromate, iodate, ammonium persulfate, and sodium perborate is certainly only circumstantial evidence as applied to flour. Furthermore, the fact that the depression of the nitrogen solubility, due to the addition of bromate, increases very considerably if the extraction is carried out on wheat flour made strongly proteolytic by the addition of papain does not provide an explanation of the extent of the effect of the proteolytic enzymes of the flour itself. In regard to the argument (7), it is quite true the glutathione activates the proteolytic enzymes. However, it is still an open question whether or not a direct chemical effect of sulfhydryl compounds might be more responsible. In addition, although it can be demonstrated that a reducing medium is present in dough, we have never been able to secure a positive nitroprusside test on a fermenting dough.

In the third main section of this book Jørgensen critically evaluates the work of other investigators in this field and frequently uses their data to support his own beliefs, although this may not always be justified. One of the papers most kindly reviewed was from this laboratory and there are a few points in regard to Jørgensen's criticisms of this paper that should be mentioned. We stated that the detrimental effect of wheat germ on the baking quality of flour was due largely to its content of glutathione. But Jørgensen says, after describing an experiment wherein wheat germ but not glutathione liquefied gelatin, "Thus it is absolutely proved that wheat germ, besides glutathione, contains a proteinase, and it would be wrong to assume that the germ's proteinases do not participate at all in wheat germ's destruction of

the baking strength. Considering the large glutathione content which Sullivan and co-workers found in the wheat germ, it is believed, though, that a large part of the detrimental effect of wheat germ must be attributed to the glutathione content." There is no argument whatever that wheat germ contains proteolytic enzymes; it is a well-known fact. The point brought out in our paper was that a water extract of germ (containing both glutathione and proteolytic enzymes), which was boiled for 10 to 15 minutes, still had a bad effect and boiling surely inactivated the proteolytic activity. This behavior can be demonstrated by farinograph curves and actual baking tests, as was shown in an article published in *Cereal Chemistry*, **14**: 489–495 (1937). Again, in the original article which Jørgensen reviewed, we stated, "It is interesting to observe in this connection that cysteine, which contains the specific -SH group, causes a very marked deleterious action as measured by the farinograph. However, unlike glutathione, cysteine permits the production of a normal loaf of bread." Jørgensen then proceeds to show that cysteine, when used at the rate of 100 mg per 560 g of flour, produced a loaf 20% smaller than the loaf without cysteine. In all our experiments cysteine caused a pronounced stickiness in the dough, as is also indicated in the farinograph curve, but when employed at the same level of -SH as glutathione, the loaves containing cysteine were practically normal in volume (only 3 to 5% lower than the standard); whereas the loaves made from flour to which glutathione was added were 20% or more below the untreated flour. Even when both compounds were added at the same level on a weight basis, provided too high an amount was not employed, the glutathione invariably gave the poorer loaf. Obviously, however, if a high enough level of cysteine is used, a poor volume will result.

Jørgensen reviewed only selected articles published to 1940, so it would be unfair to discuss in this review work that has appeared during the last five years. I wonder, however, what comments he would make on the papers published by Olcott, Sapirstein, and Blish, *Cereal Chemistry*, **20**: 87–97 (1943) and by Sandstedt and Fortmann, *Cereal Chemistry*, **20**: 517–528 (1943).

There are needless repetitions in the book and quite a little irrelevant material such as measurements of the oxidation-reduction potential of spinach juice. But I was much interested in the use of bromate in preventing the blowing of cheese.

The mechanism of the action of bromate and other improvers is a complex and intriguing problem. Whatever one may think of Jørgensen's firm conviction that the effect of certain oxidizing and reducing agents on flour is satisfactorily explained by his theory, it must be admitted that his work has contributed greatly to the solution of the problem and stimulated much needed research in this field.

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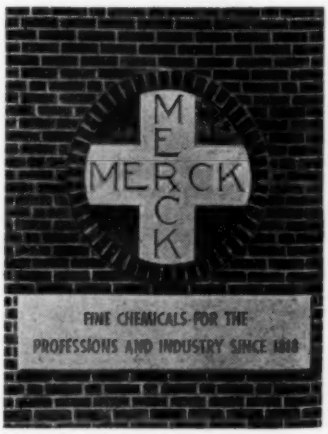

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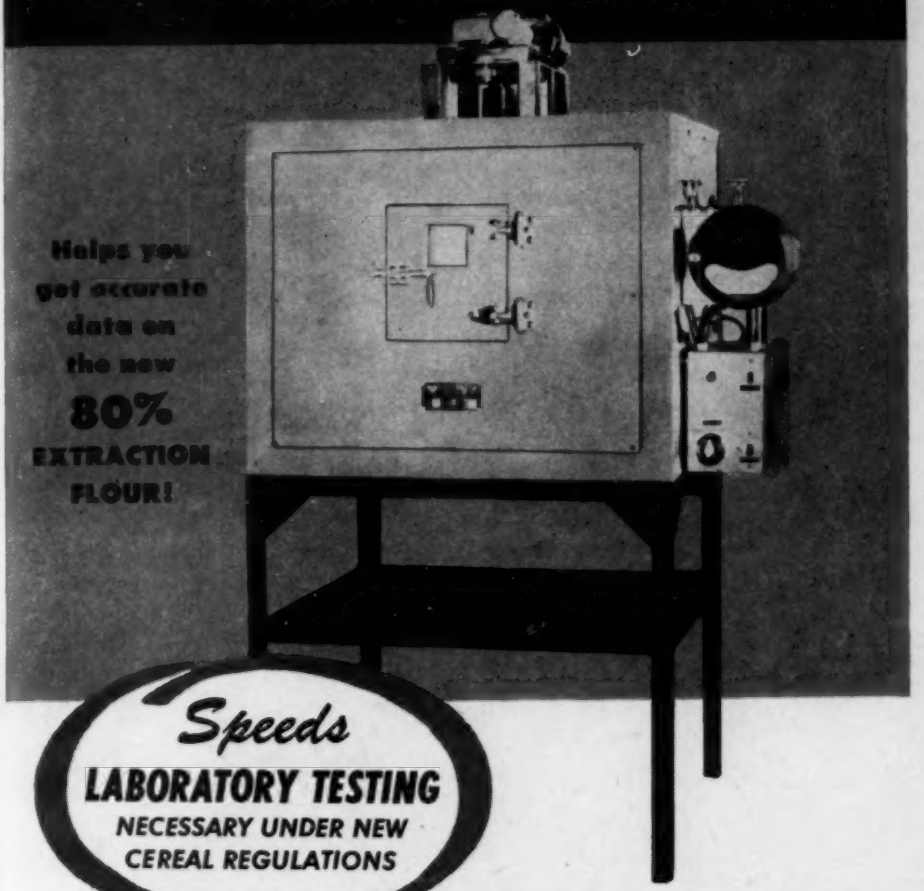

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